DEVELOPMENT OF A RESEARCH-BASED, USER-FRIENDLY, RAPID SCOUTING PROCEDURE FOR THE INVASIVE SUGARCANE APHID (*MELANAPHIS SACCHARI*), IN GRAIN SORGHUM

By

JESSICA CARRIE LINDENMAYER

Bachelor of Science in Soil and Crop Sciences
Bachelor of Science in Horticulture
Colorado State University
Fort Collins, Colorado
2013

Master of Science in Entomology and Plant Pathology
Oklahoma State University
Stillwater, Oklahoma
2015

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2019

DEVELOPMENT OF A RESEARCH-BASED, USER-FRIENDLY, RAPID SCOUTING PROCEDURE FOR THE INVASIVE SUGARCANE APHID (*MELANAPHIS SACCHARI*), IN GRAIN SORGHUM

Dissertation Approved:

Tom A. Royer

Dissertation Adviser

Kristopher L. Giles

Norman C. Elliott

Mark E. Payton

ACKNOWLEDGEMENTS

I would like to thank my amazing committee and all my friends and family for their endless support during my graduate career. I would like to say a special thank you to my husband Brad for supporting me in every way one possibly can, I couldn't have pursued this dream without you. I'd also like to thank my first child, due in a month. The thought of getting to be your mama pushed me to finish strong so you would be proud of me. Lastly, I want to thank my step father Jasper H. Davis III for showing me how to have a warrior's spirit and to never give up on something, or someone you love. Your love, spirit, and motivational words will always be heard in my heart even while you're gone.

"Where the will is strong, the way is easy."

Name: JESSICA CARRIE LINDENMAYER

Date of Degree: MAY, 2019

Title of Study: DEVELOPMENT OF A RESEARCH-BASED, USER-FRIENDLY, RAPID SCOUTING PROCEDURE FOR THE INVASIVE SUGARCANE APHID (*MELANAPHIS SACCHARI*), IN GRAIN SORGHUM

Major Field: ENTOMOLOGY

Abstract: Sugarcane aphid (SCA), *Melanaphis sacchari* Zehntner, is a significant economic pest in grain sorghum in the Southeastern US and Southern Great Plains. A collaborative project led by Oklahoma State University was tasked with developing an effective scouting plan for SCA. A stratified sampling protocol was used to collect SCA data from 299 fields from six states (OK, KS, TX, AR, LA, MS), over two years (2016-2017). Using a nested analysis of variance (NANOVA) and Taylor's (1961) power law the within field sampling variance and dispersion pattern was defined. Results from these analyses revealed two significantly different geographical regions: a southern and a northern. Results show that in either sampling region three consecutive plant samples should be taken per stop within 30m of one another. Additionally, whole plant enumerative sampling was used to define where within the plant canopy sugarcane aphids were distributed. Results from that study showed the middle of the canopy may be the best area to extract the two-leaf sample unit. Three enumerative sampling plans for estimating population density and classification of a threshold were developed. Due to large sample sizes, these sampling protocols gave evidence that a binomial sequential sampling plan would be the best option for a rapid scouting tool. To develop the binomial sequential sampling tool tally threshold regressions were analyzed to define the relationship between the mean SCA per leaf and proportion of plants infested. After the fitness and practicality of the model was considered, tally thresholds of 50 and 100 aphids per plant were selected. Wald's sequential probability ratio test (SPRT) was used to determine stop lines for both sampling plans, which ranged from 10-24 plant samples per sampling event, with an average of 11 plant samples per sampling event, depending on state, action threshold, and error level. The binomial sampling plans were validated using 48 externally sampled fields analyzed with resampling for validation of sampling plans (RVSP) software. An in-field sampling tool was developed using the tally threshold of 50 SCA. This sequential binomial sampling plan for SCA will enable time-efficient scouting, expeditiously determine if, and when an action threshold is reached, and prevent unnecessary insecticide applications.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Sorghum bicolor (L.) Moench, also known as sorghum or milo, is one of the five most important grain crops in the world (Food and Agriculture Organization 2008). In the United States alone, sorghum is a billion-dollar industry where around four million bushels of sorghum are grown on more than two million hectares annually (Elliott et al. 2017). In 2013, the sorghum industry was shaken when reports of sugarcane aphid (*Melanaphis sacchari* Zehnter) (SCA) arose as a major pest of grain sorghum on the Gulf Coast of Texas (Bowling et al. 2016). After initial reports, infestations of *M. sacchari* moved as far east as Kentucky and as far north as central Kansas (Colares et al. 2015), resulting in severe economic damage to sorghum in 17 states in the U.S. by 2015 (Bowling et al. 2016).

In response, multidisciplinary scientists have developed resistant varieties of grain sorghum, initiated altered cultural techniques, and provided chemical control options. Yet, there is still no research-based method to determine when an insecticide application was warranted, which resulted in poorly timed or unnecessary applications of insecticide. In 2015, Oklahoma State University was granted the opportunity to lead a multi-state collaborative project to define the spatial and temporal distribution of *M. sacchari* within

commercial grain sorghum, as well as develop reliable, research-based, sampling protocols designed to quickly determine when the action threshold had been met.

To build the sampling plan, the first step was defining *M. sacchari*'s dispersion pattern within a field. Using a systematic sampling protocol, 299 sorghum fields were sampled in Kansas, Oklahoma, Texas, Arkansas, Louisiana, and Mississippi. These sampling data were analysed to determine the spatial within-field sampling variance of *M. sacchari* by conducting a nested analysis of variance (NANOVA) and used Taylor's Power Law (Taylor 1961) to assign an aggregation factor. Taylor's Power Law defined two significantly different colonization patterns that were designated by geographic location and showed that the sampling pattern should begin at the field edge. The "southern" region included South Texas and Arkansas, while the "nothern" regions included Northern Texas, Oklahoma, and Kansas. The NANOVA analyses revealed that multiple samples of three plants in a row within 30 meters of one another accounted for between 80-98% of the within-field count variance.

In addition to defining sampling location within a field, multiple regression analyses were run to determine where within the sorghum canopy the two-leaf sample unit should be taken. To run these analyses, the canopy was divided into thirds catagorized as the upper, middle, and lower canopy using whole plant enumeration counts that were taken within every sampling event listed above. To better understand the relationship between the upper, middle, and lower canopy and the total aphid population per plant, state and growth stage were also included in the analysis. Results from this study showed that regardless of the state or growth stage, the middle of the plant canopy had a trend of being most predictive of the whole plant population. However, due to correlation, more models are needed to improve the confidence

of the results, the studies results are powerful enough to suggest the two-leaf sample for any of the developed sampling protocols should come from the middle of the plant canopy.

After defining "how" and "where" to sample, enumerative sampling plans were developed for *M. sacchari* in commerical grain sorghum. The first enumerative sampling plan developed was Green's (1970) fixed precision minimum sample size protocol for defined high, medium, and low economic thresholds, as well as sliding average aphid per plant curve. The results showed that at a precision level of 0.10 both the northern and southern region would require hundreds of samples to determine if a population was at a critcal mean. At 0.25 the northern region still required over 100 samples; whereas, the southern region at a higher mean per plant intensity needed as few as 41 plant samples.

Next, two enumerative sequential sampling plans were developed for population density estimation, and population catagorization based on high, medium, and low economic thresholds. Green's (1970) formula was used to develop the stop-lines for population density estimation which had similar results to the fixed precision minimum sample size calculation. This sequential sampling protocol was validatated using 50 external sampling events using resampling for validation of sampling protocols (RVSP) software (Naranjo and Hutchison 1997). The average results of the validation where higher in plant samples than Green's formula predicted, but overall the data seemed to match when the average sample number (ASN) curves were evaluated.

Lastly, for catagorization of a population above or below an economic threshold, Wald's (1947) stoplines for a negative binomial population distribution were used. Based on Wald's models, a minimum sample size from 20 plants would be needed before a decision

could be made, and similar to the other two sampling plans described, many samples were needed, especially if taken from the northern region, to make a treatment decion. The three enumerative sampling plans that were developed were not practical for use on a management level because they required too many samples to achieve an acceptable level of precision. Although, in the southern region, a sequential sampling protocol may be practical at high densities, these models largely demonstrated the need for a binomial sequential sampling protocol.

A sequential binomial sampling plan was developed to expedite monitoring for treatable *M. sacchari* intensities. First, predictive tally threshold models were evaluated for goodness-of-fit using linear regression. After carefully considering model fitness and practicallity, tally thresholds of 50 and 100 aphids per plant were selected to best predict the mean SCA per field. Wald's sequential probability ratio test (SPRT) was used to generate stoplines for sampling events (Wald 1947). Both sampling plans were then validated using resampling for validation of sampling plans (RVSP) software (Naranjo and Hutchison 1997) that provided the operating characteristic and average sample number (ASN) for 48 externally sampled fields. Averages were collected from the 500 sampling itterations, with four different action thresholds, and two error rates. The ASN ranged from 10-24 plant samples with an average of 11 plant samples.

As this project moves forward, consultants and producers will be introduced to the new sampling system that will be integrated into a smartphone application designed to fit varying regions and yield projections. This scouting system will provide a substantially more efficient and reliable SCA scouting tool for the end user. Providing user-friendly monitoring

systems will improve the likelihood of producer monitoring resulting in the elimination of unwarranted insecticide use, and the preservation of environment and effective chemistries.

CHAPTER II

REVIEW OF LITERATURE

Sorghum

Sorghum bicolor (L.) Moench (Poales: Poaceae), commonly referred to as sorghum or milo is drought and heat tolerant and is considered one of the five most important crops in the world (Sorghum Checkoff 2017). Like that of its closest relatives, corn, sugarcane, and switchgrass, sorghum is a C4 grass which allows it to be grown in many different environmental extremes (Soreng et al. 2015). Sorghum is grown in most countries around the world with the United States being the number one producer of sorghum exports (FAO 2008).

Within the United States, sorghum is grown from south Texas to South Dakota in what is commonly referred to as the "sorghum belt" (National Sorghum Producers 2016 , Sorghum Checkoff 2017). From 2014-2016, an average of 2.74 million hectares of sorghum were harvested in the United States and Kansas, Texas, Oklahoma, Colorado and South Dakota were the top five production states (USDA National Agricultural Statistics Service 2017).

In the United States, sorghum is used for livestock feed, ethanol production, and for human consumption (Carter et al. 1989, Sorghum Checkoff 2017). There are four main categories of sorghum grown in the United States: grain, forage, sugar/sweet, and biomass sorghum. Grain sorghum is the most common variant of sorghum grown in the U.S (Carter 2017). In the U.S., grain sorghum is used mainly as a livestock feed, but worldwide, sorghum grain is exported for human consumption where it is milled into an antioxidant rich, gluten free flour for breads, pasta, and beer (Sorghum Checkoff 2017).

Forage sorghum is grown solely for livestock feed and can be grazed, baled, or chopped for silage (Sorghum Checkoff 2017). Sugar sorghum, also called sweet sorghum, is grown as a source of sorghum syrup predominately in the southern U.S., but production extends into Wisconsin and Minnesota (Wittgreve 2017). This sorghum syrup was previously the most popular household sweetener but today serves mainly as an additive to some livestock grain or is used to produce whiskey (Sorghum Checkoff 2017). Like biomass sorghum, the sorghum syrup-byproduct is used to produce bio-fuel (Sorghum Checkoff 2017).

Sorghum Production in Oklahoma

Worth around $3.1 billion annually, cow-calf production is the largest agricultural commerce in the state of Oklahoma followed by hog production ($831.2 million) and broiler production ($561.6 million) (USDA National Agricultural Statistics Service 2018, Bertone 2018). Of the non-livestock agricultural commodities, grain sorghum falls just outside the top ten crop commodities produced in Oklahoma and is worth $75 million annually (USDA National Agricultural Statistics Service 2018). Nationwide, Oklahoma fluctuates between the third, fourth or fifth highest producer of grain sorghum, making

grain sorghum a highly valued commodity in the state (USDA National Agricultural Statistics Service 2017).

Grain sorghum in Oklahoma is predominately a dry-land cropping system that can have up to two plantings per growth season (National Sorghum Producers 2016, Lofton 2019). Sorghum can be double-cropped following winter wheat but is more commonly grown as a full-season summer crop in rotation with other summer crops such as soybeans, corn, or cotton. Planting dates range from mid-April to the first week of July, with anything planted later than 1 June considered "late-planted" (Hawkins et al. 2017). The most common seeding rates recommended for Oklahoma range from 60,000 to 250,000 seeds per hectare, typically on 76.2cm rows (Hawkins et al. 2017). Sorghum, provides a slightly lower return in Oklahoma ($840.00/ha) compared to other summer crops like cotton ($1,307.50/ha), and corn ($1,343.90/ha), but slightly more than soybean ($737.50/ha), making profit margins tight (USDA National Agricultural Statistics Service 2018). For this reason, input levels vary dramatically depending on projected commodity prices and producer goals. Independent of forecasted commodity prices, producers tend to invest the highest percent of the input budget on a high yielding variety with good insect and disease resistance (Luper et al. 2009). Another crucial input is fertility. The first fertilizer application is a preplant application, a critical part of seedbed preparation to ensure stand establishment, followed by a second side-dress fertilizer application in the late vegetative to early flowering stage intended to support the plant during grain fill (Hawkins et al. 2017).

Producers decisions on pesticide applications depend on pest intensity and cost of treatment (Luper et al. 2009). Weed pests pose a significant challenge for grain sorghum

production. A pre-emergent herbicide that prevents weed germination in the seed bed is crucial to ensuring the highest yield possible (Hawkins et al. 2017). Because commercial grain sorghum has no transgenic herbicide resistance traits, herbicide applications during the growing season are not common (Luper et al. 2009). The only other herbicide treatment is post-harvest to reduce johnsongrass stands, a major weed pest in grain sorghum (Luper et al. 2009). Beyond seedling stage, sorghum has very little disease pressure in Oklahoma. A fungicide seed treatment coupled with an established beneficial crop rotation generally is all that is needed (Luper et al. 2009, Hawkins et al. 2017). The main insect pests that may require an insecticide application in grain sorghum include aphids, sorghum midge, and headworms (Royer et al. 2018), and will be discussed in further detail.

The timing of grain sorghum harvest in Oklahoma largely depends on environmental conditions. If fall precipitation is too frequent and a killing frost is delayed, a harvest-aid is often necessary to dry down the residual plant material of a mature sorghum plant in order to harvest (Luper et al. 2009). Grain is harvested with a combine using a wheat header. Many farmers, to avoid excessive wear to the machinery, strategically place the corn header higher on the plant and process the heads, leaving the remaining stalk in the field. For this reason, varieties that produce tall, even height plants with elongated peduncles are more popular (Luper et al. 2009).

Important Arthropod Pests of Sorghum in Oklahoma (General Review)

Grain sorghum in Oklahoma is host to several economically important arthropod pests. After the seed is planted and through early growth stages, economically important pests include aphids, chinch bugs, cutworms, grasshoppers, lesser cornstalk borer, white

grubs, and wireworms (Royer et al. 2018). Most of these pests cause damage by feeding directly on the seed, roots, or emerging seedling. Grain sorghum treated with a neonicotinoid seed treatment before planting can reduce the chances of these pests rising to treatable levels; however, in exceptionally favorable environmental conditions all pests mentioned can cause severe yield loss.

Foliar and vegetative pests include aphids, chinch bug (especially in late planted crop during dry years), grasshoppers, lesser cornstalk borer, southwestern corn borer, and spider mites (Royer et al. 2018). These pests are generally considered sporadically economically important in Oklahoma. Most annual minor pests in the vegetative growth stages like spider mites have comparatively high economic thresholds due to the plants' ability to outgrow most vegetative damage. Others, like the lesser cornstalk borer, can cause severe damage when present in large numbers but do not regularly occur and need favorable environmental conditions to rise to treatable levels (Royer 2018).

Pests that infest sorghum from flowering through seed-fill can always become major economic pests at the proper intensity. Pests such as false chinch bugs, stink bugs, leaf-footed bugs, and sorghum webworms that feed directly on the panicle and seed can be devastating under outbreak conditions. However, because they rarely exceed established treatment thresholds, they are seldom controlled with insecticides in sorghum. Two of the most important direct pests (pests that feed directly on the developing grain) include the sorghum midge and the headworm complex. Sorghum midge, *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae), is a tropical to sub-tropical pest that has been known to be a major pest especially in the more southern regions of the U.S. (Michaud 2013). The fly causes damage by laying eggs in the flowering sorghum head

that then develop into tiny larva that consume the entire fruit (Royer 2018). Due to the nature of the feeding by sorghum midge, scouting and timely insecticide application is necessary especially in states like Texas and Louisiana where intensity exceeds treatment thresholds annually (Royer 2018).

Referred to commonly as "headworms" this pest complex is comprised of three lepidopteran species including corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuiidae); fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuiidae); and occasionally, sorghum webworm, *Nola sorghiella* Riley (Lepidoptera: Nolidae) (Matocha et al. 2008, Royer et al. 2018). Like sorghum midge, these species also feed directly on the developing grain head causing significant yield loss in high enough intensity. As with most migratory pests, intensity varies from year to year, but an insecticide treatment for headworms is warranted annually in Oklahoma and much of the southern region of the U.S. (Royer 2018).

Aphid Pests of Grain Sorghum

Aphid pests (Hemiptera: Aphididae) of grain sorghum include corn leaf aphid, *Rhopalosiphum maidis* (Fitch); greenbug, *Schizaphis graminum* (Rondani); sugarcane aphid, *Melanaphis sacchari* (Zehntner); and yellow sugarcane aphid, *Sipha flava* (Forbes).

Rhopalosiphum maidis has a dark-blue to black head with a blue-green abdomen and black legs, cornicles, and antenna. *R. maidis* is an annual, early colonizer of the grain sorghum whorl and is not known to cause significant economic damage in grain sorghum (Royer et al. 2018). *R. maidis* arrives on sorghum in early spring and stays in vegetative growth of corn and sorghum throughout the growing season. Ten or more generations can

occur during a growing season (Royer et al. 2018). *R. maidis* is known to reproduce both asexually and sexually. Most of the sexual reproduction occurs in early spring and late fall when it's cool and it reproduces asexually all summer long when populations are at a peak (Michels and Matis 2010). *R. maidis* is confirmed to overwinter in Texas but migrates into Oklahoma in the early spring.

Rhopalosiphum maidis is known to transmit several viruses to corn making it a more substantial pest in that crop than in sorghum (Royer et al. 2018). In fact, to the opposite effect, *R. maidis* has been studied as an early food source for many natural enemies resulting in increased biological control of *S. graminum* by natural enemies (Kring and Gilstrap 1986, Michels and Matis 2010). Today, based on high observed levels of parasitism and predation, it is thought that *R. maidis* may have a similar impact on low populations of *M. sacchari* as well (Hewlett et al. 2019). Overall, this aphid may colonize and feed on sorghum whorls but has not shown to cause damage and has been reported as an early food source for natural enemies that help reduce intensities of damage causing insects like *S. graminum* and *M. sacchari* (Kring and Gilstrap 1986, Michels and Matis 2010).

Sipha flava is an easily distinguishable aphid with bright yellow color and short black hair-like spines in rows down its abdomen (Blackman and Eastop 2017). In addition to these stand-out features *S. flava* has yellow legs, antenna, and cornicles, and the cornicles are so small they are almost invisible to the naked eye. *S. flava* arrives on the lower leaves of sorghum in late spring early summer and each female can produce up to 58 clonal offspring (Royer et al. 2018). Besides grain sorghum, *S. flava* can reproduce

on many native North American grasses because it is believed to be indigenous to North America (Blackman and Eastop 2017).

Sipha flava causes the most damage to early stage sorghum (V1-V4) on the first or lower leaves (Blackman and Eastop 2017). When *S. flava* has been feeding, the leaves turn an inimitable purple followed by lasting chlorosis. If the population continues to feed and grow damage can result in leaf death and senescence (Blackman and Eastop 2017). Yet, in Oklahoma *S. flava* consistently arrives on grain sorghum in the late stages of vegetative growth leading to very little cumulative damage to the plant. For this reason, *S. flava* is not considered an economic pest in grain sorghum in Oklahoma (Blackman and Eastop 2017).

Schizaphis graminum commonly referred to as "greenbug", was historically a key pest of wheat after its initial outbreak in Virginia in 1882 (Brewer et al. 2019). While sorghum was a known host for *S. graminum*, it did not become a prominent pest of sorghum until 1968 (Royer et al. 2015). Today, *S. graminum* is still a frequent colonizer on many grain crops but largely, thanks to host-plant resistance and insecticide seed treatments, is an occasional pest on sorghum throughout the central Great Plains (Brewer et al. 2019).

Schizaphis graminum is described as a light green aphid with a dark green stripe down its abdomen, legs are light green with dark green tarsus and dark green cornicles. Unlike *S. flava* and *R. maidis*, *S. graminum* overwinters in Oklahoma on winter wheat (Royer et al. 2018). Once winter wheat senesces, the winged morphs migrate to sorghum in the late spring and early summer. *S. graminum* damage to sorghum has a similar appearance to rust, causing reddish-yellow freckling on the surface or ventricle side of

the leaf. Although *S. graminum* is no longer an annual economic pest of grain sorghum, monitoring populations is still important. Economic thresholds for *S. graminum* in sorghum are based on growth stage and require sampling 25-50 plants for both the aphid and the parasitized mummies (Blackman and Eastop 2017, Royer et al. 2018, Brewer et al. 2019).

Sugarcane Aphid (*Melanaphis sacchari*)

Description and Biology

Melanaphis sacchari is often decribed as being crème to "lemon" (Singh et al. 2004) in color with dark brown cornicles, antennae, legs, and tarsi (Bowling et al. 2016). Alate, or winged adults, similar in color with the addition of a black patch or pattern on the dorsal scleritis (Eastop 1955, Blackman and Eastop 2000). A colony is primarily made up of parthenogenic, viviparous, apterous and alate females (David and Sandhu 1976) ranging between 1.1-2.0mm long (Blackman and Eastop 2000). A single adult female can produce as many as 96 nymphs in 37 days (Blackman and Eastop 2000). Immature females (nymphs) grow through four instars in 4.3 to 12 days before becoming reproductive (Royer 2016).

Although aphids typically go through ten to thirty generations of asexual clones in a year, perhaps over 30% of aphid species also have a single sexual cycle at the end of summer as fall approaches (Dixon and Kundu 1998, Caillaud et al. 2002b). Using photoreceptors between the antennae called ocelli, aphids respond to seasonal changes like cooler temperatures and shorter photoperiods (Dixon and Kundu 1998, Caillaud et al. 2002b). Species that have a single sexual cycle are referred to as portraying cyclical parthenogenesis (Ogawa and Miura 2014). They can be monoecious (one host) or heteroecious (two different hosts). Evolutionarily, cyclical parthenogenesis is

hypothesized to have originated around 200 million years ago and is considered to be a more primitive reproduction method than the strictly clonal parthenogenesis seen in later evolving species (Delmotte et al. 2002, Ogawa and Miura 2014). Species associated with a sexual cycle thought to originate from the more northern latitudes in order to produce an egg, which serves as an overwintering survival strategy.

The shorter photo-phase of an oncoming fall triggers endocrine system signals that enable the aphid to produce male and female sexual morphs. Typically, males are produced in the first generation followed by oviparous females coming later as hormone titer continue to become more intense (Ishikawa et al. 2012). These sexual morphs mate and produce a very cold hardy egg that then hatches a "fundatrix" or "stem-mother" in the spring, when conditions are more favorable (Ogawa and Miura 2014). This fundatrix female is asexual and goes on to produce more viviparous females. At this time, there is no evidence that *M. sacchari* undergoes cyclical parthenogenesis in North America, thus it functions as a viviparous parthenogenic aphid with an anholocyclic lifecycle that remains on its grass hosts all year long (Nibouche et al. 2014, Bowling et al. 2016).

Wing development for aphids is a fundamental survival tactic that allows the insect to disperse and further the continuation of its life cycle. It is thought that 95% of aphid species have the ability to generate winged morphs (Braendle et al. 2006). Including elements of morphological, behavioral, chemical physiological change, the winged morph is collective a response to the environment that is thought to be brought on by both exocrine and endocrine hormone signals triggering gene-expression (Braendle et al. 2006, Ishikawa et al. 2013, Zera 2016). As biotic or abiotic stresses increase, the mother aphid produces some offspring with the capacity to develop wings. This winged

morph is then able to relocate and continue asexual reproduction. Though the exact mechanism is still unknown, researchers believe the aphid responds to environmental stimuli through mechanoreceptors and olfactory receptors that sense stresses like overpopulation and reduced plant nutritional value (Hardie et al. 1996, Caillaud et al. 2002a, Guo et al. 2016). These stimuli activate neurosecretary brain cells, which trigger a cascade of signals that result in hormone expression. Hormone expression is thought to cue development of winged morphs within the viviparous female. When the migrating alatae land on a suitable host crop, they will begin producing nymphs to perpetuate the lifecycle. *M. sacchari* is believed to overwinter on plant hosts in southern latitudes then migrates to northern latitudes during summer where it recolonizes sorghum (Brewer et al. 2019, Bowling et al. 2016, Blackman and Eastop 2000).These northern migrations are completed by the alate or winged morphs (Bowling et al. 2016, Blackman and Eastop 2000).

Population genetics structure and world distribution

The host shift by *M. sacchari* that occurred from sugarcane to sorghum in the U.S. generated speculation that there may be more complexity to the species lineage than what is expected of a presumably clonal species. Aphids as a whole display varying levels of host specificity with less than one percent of aphid species being generalists (Peccoud et al. 2010). *M. sacchari* undergoes apomictic parthenogenic reproduction meaning the oocyte replicates mitotically resulting in two diploid daughter cells (Bermingham and Wilkinson 2009). Although this method of reproduction is highly efficient, it results in restricted genetic recombination, which in turn limits geneticists ability to define clear lineages and make definitive conclusions on why this aphid became

such a severe pest in sorghum in 2013 due to highly clonal lineages (Nibouche et al. 2015).

Early reports of *M. sacchari* in grain sorghum speculated that it was a different race of aphid entirely, previously described by the name *Melanaphis sorghi* (Theobald) (Brewer 2013). This hypothesis stemmed from earlier work which described *M. sorghi* as the same species as *M. sacchari*, but was exclusively host specific to sorghum varieties (Remaudiere 1996). A world-wide population genetics study (Nibouche et al. 2014) was conducted consequently following the first reports of *M. sacchari* being a major pest of grain sorghum in the U.S. Authors concluded that there were five distinct multi-locus lineages (MLLs) displaying robust correlation to geographic location. The aphid haplotypes where broken down as follows: MLL A-West and East Africa, MLL B-Australia, MLL C-South America, the Caribbean, East Africa, and the Indian Ocean, MLL D-the United States, and MLL E-China (Nibouche et al. 2014). Though this study gave conclusive evidence to geographic evolution, the author admits to its limitations on solving the host specialization question.

In 2014, Nibouche et al. (2014) demonstrated that two haplotypes were associated primarily with sugarcane, while the third haplotype was only present on wild type sorghum, *Sorghum bicolor spp. verticilliforum* (L.) Moench (Poales: Poacea). This study provided strong evidence of genetic differences in population structure based on host preference. In the follow-up study (Nibouche et al. 2015), these computationally observed genetic differences were tested using laboratory transfer experiments. Based on differences in survivorship they exhibited when grown on the two host plants, the sugarcane and sorghum haplotypes remained consistent. However, a third haplotype

arose that exhibited no significant difference in development when reared on either sorghum or sugarcane and was coined the "intermediate" haplotype (Nibouche et al. 2015). Combined, these two studies portray a story of evolution based on species fitness in a given environment and supports evidence to a second hypothesis of a host shift rather than new invasion of a more virulent race of *M. sacchari*. However, the findings in 2014 and 2015 seem to have only been the beginning of the story since the latest study (Nibouche et al. 2018) found evidence for the introduction of a new genotype cluster (MLL F) within the defined haplotypes previously described. Like many evolutionary narratives, especially in highly clonal species, there can be multiple paths to genetic variance led by both immigration of new genotypes and mutations of the existing population (Milgroom 2017).

History, Distribution, and Current Pest Status in the U.S.

Originally described by Zehntner in 1897, *M. sacchari* is an evolutionarily opportunistic species that's pest status seems closely affiliated with the domestication and spread of its two predominate crop hosts; sugarcane, *Saccharum officinarum* L. (Poales: Poacea) and sorghum. (Singh et al. 2004). *M. sacchari*, also reported as *Aphis sacchari* and *Longiunguis sacchari*, has been found on a wide range of grasses in 25 countries across Asia, Africa, North America, and South America (Singh et al. 2004).

Melanaphis sacchari's presence was first reported in Florida in 1922 (Wilbrink 1922) and was considered a minor pest of sugarcane by 1977 (Summers 1978, Denmark 1988). In 2013, it was reported infesting sorghum in the Gulf Coast of Texas. (Bowling et al. 2016, Armstrong et al. 2015, Colares et al. 2015a, Nibouche et al. 2014). Following this initial finding, sugarcane aphid was discovered infesting grain sorghum in all of

Texas, Louisiana, Oklahoma and Mississippi. During the following year (2014) the heavy infestation of sugarcane aphids moved eastward into Kentucky and north into central Kansas (Colares et al. 2015a, Armstrong et al. 2015). By 2015, the aphid spread to 17 states and 400 counties in the United States (Bowling et al. 2016). *M. sacchari* is not the first invasive exotic species of aphid, an important example being Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae), nor is this the first aphid that "switched" hosts to become a pest of a crop that was not previously affected. *Schizaphis graminum* switched from its first reported economic host, wheat, to sorghum (Harvey and Hackerott, 1969)

There are three historically based stages of naturalization well summized and defined by (Colares et al. 2015) the epidemic stage: this is described by the wide geographical distribution, tremendous population numbers, and severe crop damage; 2) the attenuation stage: infestations become more limited to certain geographical ranges, and the severe outbreaks become more sporatic; finally 3) the endemic stage: when the outbreaks that cause severe economic damage become rare due to the onset of natural ememies adapting to this new prey, a cycle only to be disrupted by a sudden change in environment due to natural or cultural events (Colares et al. 2015a). It is only with sound integraded pest management strategies like host plant resistance, chemical and cultural control, that stage three can be attained.

Infestation Patterns and Harvest Difficulty

Melanaphis sacchari colonizes new locations when alates (winged forms) are wind disseminated by seasonal wind patterns moving from south Texas and Mexico towards more northern latitudes (Blackman 2000, Bowling 2016). The infestation pattern

on the plant can vary greatly from geographical location and growth stage. Aphids begin an infestation by landing on the host then colonizing the underside of the lower leaves where they increase rapidly from flowering to grain-fill until they have consumed the entire plant (Armstrong et al. 2015). Lab observations have described *M. sacchari* feeding as intermediate all the way through to sexual maturity (Armstrong et al. 2015, Elliott et al. 2015).

Melanaphis sacchari causes yield loss on all the sorghum variants including grain, forage, sugar, and ethanol producing (Singh et al. 2004, Armstrong et al. 2015, Bowling et al. 2016, Sorghum Checkoff 2017). Yet, *M. sacchari* has been reported as less physically damaging to the photosynthetic capabilities of the plant when compared to some of its counterparts like *S. graminum*. It requires many more aphids feeding for a longer period to achieve the same chlorotic symptoms expressed by *S. graminum* in sorghum (Bayoumy et al. 2016). However, as a result of high quantities of honeydew produced by *M. sacchari,* sooty mold, caused by various Ascomycete fungi begins to grow on all the leaf surfaces in some cases covering them completely (Royer et al. 2016). Sooty mold covering the leaves leads to complications with harvest machinery and may lower photo synthetic capabilities for the plant (Bowling et al. 2016, Royer 2016).

Current Integrated Pest Management of *M. sacchari*

Cultural Control

Cultural control research is limited at this time; however, it is recommended that producers, without over-wintering populations, plant as early as possible and reduce the amount of johnsongrass around their field (Royer 2014, Buntin 2015). Earlier planting dates allow the sorghum plant to be further developed when the sugarcane aphid arrives

lowering the number of times insecticide applications must be made if at all. Removing or limiting the amount of johnsongrass will help remove a secondary host for the aphid to reside in before moving onto the planted sorghum. Most important to properly controlling *M. sacchari* populations is to scout and monitor populations as often as possible once the aphid has been reported in the area (Royer 2014, Buntin 2015, Bowling et al. 2016, Brewer and Gordy 2016). Developing a research-based population estimation protocol is critical to uniting the front against high infestations of sugarcane aphid.

Melanaphis sacchari was demonstrated in the field to colonize and proliferate on 15 different varieties of sorghum, at varying rates, and johnsongrass (Armstrong et al. 2015). Besides sorghum and sugarcane, *M. sacchari*'s early perceived suitable hosts consisted of many members of the grass family (Poales: Poaceae). They initially included corn *Zea maize* L., teff grass, *Eragrostis tef* (Zucc.) Trotter, winter wheat, *Triticum aestivum* L., rye, *Secale cereal* L., barley, *Hordeum volgare*, L., and proso millet, *Panicum miliaceum*, L. Because *M. sacchari* can use so many grass species in the same tribe as hosts, there is a large economic incentive to identify tolerant varieties that reduce yield loss. Fortunately, Armstrong et al. (2015) demonstrated, when infested at the 3-4 leaf vegetative stage in the greenhouse, *M. sacchari* aphids currently infesting sorghum in the U.S. not only failed to colonize, but experienced 100% mortality in all but the species closely related to sorghum like johnsongrass.

Bayoumy et al. 2016 screened *S. graminum* resistant sorghum lines for *M. sacchar*i resistance, hypothesizing that they may have some cross-resistance. This hypothesis was corroborated in a greenhouse study when different lines holding the trait 'PI 550610' showed robust signs of antibiosis against *M. sacchari*. The resistant lines

were used as a starting point for future screening (Armstrong et al. 2015). In another lab study, sorghum lines with 'PI 550610' gene showed varying levels of antibotic resistance to immature *M. sacchari* while delaying development and slowing the reproductive rate in comparison to a susceptible variety (Bayoumy et al. 2016). However, there are currently large inconsistancies surrounding the definition of tolerance in regards to this pests economic injury level as well as field variety screenings are varing greatly with the geographic location and the growth stage upon infestation (Bayoumy et al. 2016, Armstrong et al. 2015). This inevitably will be a ongoing endeavour not far from what was seen in the 10 years of variety selection to control the populations of *S. graminum.*

Biological Control

Grower response to a new invasive species such as *M. sacchari* often includes over application of insecticides, which potentially impedes natural enemy establishment (Stern et al. 1959). If chemical control can be confined to the most efficient intervals for control, there is the potential for natural enemies to become a greater contributing factor for *M. sacchari* control. As *M. sacchari* infestations increased in intensity, Kansas researchers documented increases in natural enemy numbers, similar to that reported for *S. graminum* in the field (Colares et al. 2015b). Native species like *Hippodamia convergens* (Guerin-Meneville) (Coleoptera: Coccinellidae), *Colemegilla maculata* (DeGeer) (Coleoptera: Coccinellidae), a complex of Chrysopid species that includes *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), and *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), are identified as potential contributors for natural control of *M. sacchari* under low-density populations. In a greenhouse study that compared the feeding and development of these four predators on *S. graminum*, *M. sacchari*, and

Ephestia. *Kuehniella* (Zeller) (Lepidoptera, Pyralidae), the four natural enemies that were adapted to *S. graminum* consumed and developed just as well, if not better, on *M. sacchari* (Colares et al. 2015a).

Chemical Control

Currently there are two commonly recommended foliar insecticides for *M. sacchari* on sorghum. The most efficacious insecticide is Sivanto® (Bayer CropScience, Leverkusen, Germany, 17.09% flupyradifurone) a sub-group 4D (IRAC 2018) insecticide that is recommended at a rate of four ounces per acre rate and has a pre-harvest interval time of twenty one days (Royer 2014, Buntin 2015, Bowling et al. 2016, Brewer and Gordy 2016). The other insecticide is Transform® (Corteva, Indianapolis, IN, 50% sulfoxaflor) which is a sub group 4C (IRAC 2018) insecticide that is recommended at a rate of one fluid ounce per acre and has a pre-harvest interval of fourteen days (Royer 2014, Buntin 2015, Bowling et al. 2016, Brewer and Gordy 2016). This insecticide is not currently fully labeled and needs a Section 18 Emergency Use Exemption (EPA 1111) in the state to be legal to apply. Products containing the organophosphate insecticides chlorpyrifos and dimethoate have also shown to have activity on *M. sacchari*, but because they require long per-harvest intervals, are less target specific and less efficacious, thus they are not as widely recommended (Buntin 2015). Pyrethriod insecticides are not recommended and have been shown to actually flare sugarcane aphid populations (Royer 2014, Buntin 2015, Bowling et al. 2016, Brewer and Gordy 2016). Insecticidal seed treatments have also proven to be effective in controlling *M. sacharri* populations for up to 40 days (Royer 2014, Buntin 2015). The three top registered

neonicotinoid seed treatments are products containing any one of the following active ingredients thiamethoxam, clothianidin, or imidacloprid (Buntin 2015).

Monitoring, Sampling Strategies, and Economic Thresholds

Integrated pest management is a multi-disciplinary, individualized production management system that works within an agroecosystem to combine biological, cultural, mechanical and chemical control in order to maintain a pest population below economic threshold (Norris et al. 2003, Stern et al. 1959). This concept can be thought of as a triangle with base and mid-layers made up of biological control components including; natural enemies, host plant resistance, and cultural techniques like tillage, irrigation, refuges, and planting dates (Stern et al. 1959). The smallest part of the triangle, at the very top, would be chemical control; which is to be used efficiently and sparingly (Norris et al. 2003). The foundation of a successful integrated pest management system is appropriate scouting and monitoring of pest populations. Accurately monitoring pest populations determines when the pest has met the economic threshold and whether or not chemical application is necessary (Stern et al. 1959, Norris et al. 2003a). The economic threshold is a research based population density at which action should be taken to deter the population from reaching the economic-injury level where yield loss occurs (Stern et al. 1959).

Currently, there is no research-based method to determine when a field has *M. sacchari* pressure that exceeds an economic threshold (ET). Early sampling recommendations for *M. sacchari* were rudimentary and based spray decisions on a percent of plants with substantial honeydew on them (Catchot et al. 2015). Two more recent *M. sacchari* sampling protocols are based on an intensity per-leaf economic

threshold or by a growth stage "threshold". The per-leaf threshold sampling protocol directs the scout to estimate the aphid intensity on an upper most and lower-most leaf on ten randomly selected plants, in four different locations within a field (Biles 2018). If the average *M. sacchari* per leaf is more than 50, the recommendation is to chemically treat within three days (Biles 2018, Gordy et al. 2018). This sampling method seems to be based on published research by Szczepaniec (2018) done exclusively in the High Plains of Texas. While the research objectives were largely to determine and yield-based economic threshold, no sampling protocol was discussed (Szczepaniec 2018). This leaves the question of why ten samples from four stops of ten plants are needed.

Another newly developed "threshold" based sampling plan based on growth stage was even more perplexing. The sampling plan recommends treatment if 20-30% of plants (based on a two-leaf sample of an upper-most and lower-most) are infested with 50 or more *M. sacchari* (Biles 2018, Gordy et al. 2018). Additionally, this sampling plan, while binomial, is not sequential and requires four stops of ten randomly selected plants making a total of 40 plant samples (Biles 2018, Gordy et al. 2018). Multiple other scouting protocols and recommended thresholds exist ranging from 40-60 samples, 20-30% infested with 25-125 sugarcane aphids per leaf or per plant (Royer 2015, Gordy et al. 2019, Royer 2016, Armstrong et al. 2017, Sorghum Checkoff 2017). Yet, there remains no refereed study that encompasses multiple states, sites, and growth stages to produce a rapid classification protocol for determining of an ET has been met for *M. sacchari.*

Sampling Strategies

Defining Dispersion Patterns

Sampling protocols used for population monitoring are most constructive when they integrate the biology of the monitored pest with the protocol. The first step to developing a sampling protocol of any kind is to define the statistical dispersion pattern of the organism sampled. There are three statistical categories of dispersion in insects; aggregated, random, and regularly distributed (Norris et al. 2003, Pedigo and Buntin 1994). Aggregated or clumped populations, like that of aphids, are pests that occur in patches that are distant from other population clumps within an area. Sampling data for this special distribution would be described as a large number of zeros with a few samples having sizable population numbers (Pedigo and Rice 2006, Norris et al. 2003). Aggregated populations often require many samples to obtain adequate information on the population.

Random- or Poisson-distributed insects are described as having their population mean equal to the population variance (Norris et al. 2003, Pedigo and Buntin 1994). In this population distribution, finding one individual likely indicates another individual may be near, but not cohabitating. Random distributions are not very common in nature but can be found in some Coleopteran and Lepidopteran species (Pedigo and Buntin 1994).

Regular distribution is even more rare in nature than random and is classified by a population where every sample unit has the same number of individuals. Regularly distributed populations which are typically found in insects that have a repulsion factor such as cannibalism and are typically predators of other insects (Pedigo and Buntin 1994). These individuals are usually equally spaced across a landscape. By finding an

individual, there is a good chance another individual will be within a calculated proximity to the last depending on biological need (Norris et al. 2003).

In order to statistically define an insect's dispersion pattern, there are multiple indices that can be used. A widely used index for negative binomial populations, like most aphid species, is the *k* index or common *k*. A common *k* value, calculated by the formula $k=m^2/(s^2-m)$ where s^2 is the variance and m is the mean, is a constant given to an insect population with a negative binomial probability distribution, to define where the population lies on an aggregation index or scale (Bliss and Owen 1958, Elliott 1977). On the common *k* scale, *k* values less than two indicate highly clumped populations where as a value greater than eight indicates an almost Poisson distribution.

While a common *k* index is indicative of how clumped a population is and is relatively easy to calculate once the probability distribution has been defined, a common *k* does have its disadvantages that may disrupt the reliability when used in a sequential sampling protocol. One of the main disadvantages is that a common *k* index can vary with mean density. For an aphid like *M. sacchari*, this may not portray the dispersion accurately during large outbreaks or when population intensity is very low (Pedigo and Buntin 1994). To put this in perspective, for *M. sacchari*, the common *k* value could change quickly based on when, where, and how the samples are obtained during a growing season; potentially changing the optimum sample size and start and stop points for the sequential sampling protocol on a weekly or even daily basis depending on environmental conditions (Pedigo and Buntin 1994). The goal of an effective sequential sampling protocol is that it is reliable and consistent, for this reason a common *k* may not

be the best aggregation factor to apply to a pest like *M. sacchari* whose population means can vary so dramatically.

To overcome the lack of consistency present in the common *k*, two empirical models were developed: Lloyd's mean crowding (1968) that uses Kuno's formula (1969) for defining sampling start and stop lines (Lloyd 1967, Kuno 1969); and the more robust and popular Taylor's Power Law (1961) that uses Green's formulas (1970) to develop optimum sampling size and sampling stop lines (Green 1970, Elliott 1977, Pedigo and Buntin 1994, Pedigo and Rice 2006). Like probability distributions, empirical models were developed to classify quantitatively an insect's population distribution within a given area. What makes empirical models stronger, in some cases, than probability distributions are their density independence. Empirical models can be more consistent spatially and temporally than probability distributions in populations with highly variable means because of being independent of the mean (Pedigo and Buntin 1994, Young and Young 1998).

As mentioned above, Taylor's power law is the most used empirical model for defining dispersion of highly aggregated populations (Taylor 1961). Taylor described that for most animals, including insects, there is a linear relationship between the base-10 log transformations of the mean and variance (Pedigo and Buntin 1994, Pedigo and Rice 2006). In other words, for every mean, regardless of size, there is a relationship to variance around that mean for any given sample set. This can be represented with the thinking formula $s^2=\alpha m^b$ where s^2 is the variance, m is the mean, α is the considered the "sampling factor" a representative of the sampling unit or sampling scale, and b is the aggregation factor associated with the animal population (Pedigo and Buntin 1994).

Aggregation factors using Taylor's Power Law are measured as ƀ=1 is a random distribution, ƀ=0 is a regular dispersion and ƀ>1 is a clumped dispersion (Elliott 1977, Leather 2009). Being able to better define highly aggregated populations made developing optimum sample sizes and sequential sampling protocols more consistent.

Enumerative Sampling Plans: Fixed Sample Size or Sequential Sampling

Enumerative sampling, in which every individual is counted, can be a highly reliable method of estimating a critical density or a total population density. When enumerative sampling is being conducted, there are two definitive ways of sampling: using a fixed sample size, or sequentially sampling using calculated stop-lines. For fixed sampling size calculations, Green's (1970) formula for minimum sample size estimation is calculated using the formula $n=\alpha m^{b-2}/D_{exp}$ where n is the estimated minimum samples, α and ƀ are coefficients from Taylor's power law, m is the mean/critical density/economic threshold, and D_{exp} is the desired level of precision (Green 1970, Elliott et al. 2003). While this formula provides the minimum number of required samples to estimate a mean at a given precision level, it is often considered impractical due to high sample sizes needed. For this reason, sequential sampling plans are more commonly used. They are more practical in that the sample size varies on the intensity of the population. In extremely high or low populations, this sampling method is often more efficient in application.

There are two main objectives for enumerative sequential sampling: to estimate a population density in a given area, or to classify a population as above or below a critical density. When estimating a population density, cumulative insect counts are used to determine when a stop-line, at a fixed precision level, has been exceeded. The widely

used sequential sampling stop-line for population density estimation was defined by Green (1970). The formula for these stop-lines is:

$$\boldsymbol{logT_n = \frac{\ln\left(\frac{D^2}{a}\right)}{b-2} + \frac{b-1}{b-2} ln}$$

Where T_n = the cumulative number of aphids sampled, n= the total number of samples, D^2 = desired precision level, and *a* and *b* = Taylor's power law parameters mentioned above. While sequential sampling for population density estimation can be very beneficial, especially in the scientific arena, in certain species it may be impractical at the field level due to how many cumulative insects must be recorded.

The last enumerative sampling plan is sequential sampling for classification of a population as above or below a critical density. This type of sampling is often used for applied purposes to make a treatment decision. The stop-lines calculated in this type of sampling predict upper and lower boundaries where treatment is necessary when the cumulative number of individuals passes the upper line, and no treatment is required when the cumulative number of individuals is below the lower line. If the cumulative number of individuals is neither above the upper line nor below a line, the sampler would continue to sample until a line is crossed. This method of sampling usually decreases the amount of time needed to determine if a population is above or below a critical density especially at very high or very low intensities because the count will cross an upper or lower stop-line quickly. At population densities around the critical density, more samples may have to be taken. There are multiple formulas to develop these stop-lines, but Wald's (1947) sequential probability ratio test (SPRT) is the best fitting model for known

distributions like *M. sacchari's.* Wald's SPRT stop-lines for aggregated population distributions are calculated using the following formulas:

$$\mathbf{d_1 = (b*n) + h_1}$$

$$\mathbf{d_2 = (b*n) + h_2}$$

$$\mathbf{b} = k * \left(\frac{ln\left(\frac{q_2}{q_1}\right)}{ln\left(\frac{p_2 * q_1}{p_1 * q_2}\right)} \right) \qquad \mathbf{h_1} = \left(\frac{ln\left(\frac{\beta}{1-\alpha}\right)}{ln\left(\frac{p_2 * q_1}{p_1 * q_2}\right)} \right) \qquad \mathbf{h_2} = \left(\frac{ln\left(\frac{1-\beta}{\alpha}\right)}{ln\left(\frac{p_2 * q_1}{p_1 * q_2}\right)} \right)$$

Where $p_1 = (m_1/k)$, $p_2 = (m_2/k)$, $q_1 = (1+p_1)$, and $q_2 = (1+p_2)$. Where k = common k and m_2 and m_1 = the ET and 1/3-1/2 of the ET. In the top, corresponding equation, d_1 =the upper stop-line, d_2 = the lower stop-line, and n = the number of plant samples. On a basis of practicality, enumerative sequential sampling can be ideal for insect populations where relative densities stay low. However, in species like *M. sacchari* where the population sizes can be very high (in the thousands of aphids), these sampling protocols may not be ideal.

Binomial Sequential Sampling Plans

While enumerative sequential sampling is predominately for research objectives, binomial sequential sampling is almost always for monitoring pest populations in an agronomic setting (Pedigo and Buntin 1994). Binomial sampling is declaring if the sample unit is "infested" or not based on the presence or absence of an individual or group of individuals rather than enumerating every individual on the sample unit (Pedigo and Buntin 1994, Pedigo and Rice 2006). Binomial sampling programs are often used in commercial agriculture IPM programs because they are very efficient in terms of time and sampling cost.

Although binomial sampling plans are highly efficient in determining when an ET has been met for direct pests, these are used more frequently for indirect pests such as *M. sacchari* (Pedigo and Buntin 1994). Unlike direct pests, indirect pests do not cause economic damage to the fruit itself but cause yield loss to the crop by reducing the crops ability grow and develop normally. An indirect pest then can have more individuals per sample unit before causing damage. For this reason, indirect pests aren't typically sampled using a classical presence or absence of an individual pest, but by developing a tally threshold. A tally threshold is an intensity that is predictive of the population mean by way of proportion of sample units infested.

To visualize this concept further, the thinking formula for binomial sampling plans are described as P_T–m relationships, where "P_T" is proportion infested with >T individuals, and T being the established tally threshold, while m is the mean of the total population. In the case of the *M. sacchari*, the tally threshold values would be derived from using the regression model described by Giles et al. (2000) that is $ln(-ln[1-PET]) = a + bln(m)$ where *a* and *b* are the predetermined coefficients from the Taylor's Power Law log base-10 regression. The T-value selected would be the value with the best goodness-of-fit judged by the r^2.

After determining the best-fit tally threshold, stop-lines would be calculated using Wald's SPRT formula (Wald 1947). Instead of a relationship between cumulative individuals and number of plant samples, the relationship would be amongst the percent of plant samples infested with T individuals and number of plants inspected. Both enumerative and binomial sequential sampling plans for classification revolve around a defined critical density or mean per plant, the largest difference being no counting is

necessary in binomial sampling. The greatest benefit to binomial sequential sampling is the reduction in time necessary to sample by eliminating the need to enumerate every aphid on the leaf or other sample unit.

Economic Injury Levels and Economic Thresholds for *M. sacchari*

Recently, the first peer reviewed study was published that determined economic injury level (EIL) based action thresholds (Gordy et al. 2019). In this study, aphid populations were placed into two categories: high population growth rate and low population growth rate. Using the population growth rate regression equations, in addition to commodity price and treatment costs, the authors determined the EIL to range from 37-102 *M. sacchari* per leaf. Adjusting to aphid intensity growth observations led to ET's that range from 19-137 aphids per leaf with the author suggesting an ET of 40 *M. sacchari* per leaf to be the best fit across multiple environments and production costs (Gordy et al. 2019). This study concludes with a statement about the need for a binomial sequential sampling plan to quickly determine when thresholds are met, ultimately leading to the need for this study.

CHAPTER III

WITHIN FIELD AND WITHIN PLANT CANOPY DISTRIBUTION PATERNS OF *MELANAPHIS SACCHARI* (ZEHNTNER) IN COMMERCIAL GRAIN SORGHUM

Abstract

Sugarcane aphid *Melanaphis sacchari* (Zehntner) is an introduced aphid species that became a substantial economic pest of grain sorghum in the United States. From previous invasions of aphid pests, we know effective monitoring and early detection are important keys to management, but such information is currently lacking in this species. In this study, 281 sampling events in 134 geographic locations across Kansas, Texas, Oklahoma, and Arkansas were used to evaluate within field and within plant canopy distribution patterns of the sugarcane aphid. Based on the results from a nested analysis of variance (NANOVA), the best sampling method to account for the most variance amongst aphid counts is by evaluating three consecutive plants in a row using a two-leaf sample unit. The consecutive sampling of three plants, or stops, should occur within a 30m radius of one another beginning at an edge and moving in an inverted "U" shape into the field. Lastly, analysis of within canopy distribution patterns revealed that leaves occupying the middle of the plant were most predictive for estimating aphid population intensity per plant. By increasing sampling precision, we can increase likelihood of early detection therefore leading to better management of this pest.

Introduction

Sorghum, *Sorghum bicolor L.* (Moench) (Poales: Poaceae) is a drought and heat tolerant grass crop, and one of the five most important crops in the world (Sorghum Checkoff 2017). The United States is the leading producer of grain sorghum, producing nearly 12 tons of grain sorghum over the last decade that generated billions of dollars in economic revenue (Food and Agriculture Organization 2008, Elliott et al. 2017, U.S. Dept. of Agriculture 2017). Sugarcane aphid *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) is a subtropical aphid that was introduced into the U.S. on or before 1922 (Wilbrink 1922). Once considered to be a minor pest of sugarcane (Summers 1978, Denmark 1988, White et al. 2001), it became an important economic pest of sorghum in the U.S. after 2013 when it was discovered in large numbers on grain sorghum in Louisiana, Texas and Oklahoma (Brewer 2013, Armstrong et al. 2015). Yield losses from sugarcane aphid infestations ranged from 45 to 181 kg/ha (Bowling et al. 2016). Sugarcane aphid rapidly expanded its range, spreading east to Kentucky and north to Kansas (Armstrong et al. 2015, Colares et al. 2015). By 2015, the aphid was reported in 17 states and 400 counties in the United States (Bowling et al. 2016). The sugarcane aphid remains a pest at some level in grain and forage sorghum in most sorghum producing states (Lagos-Kutz et al. 2018).

The objectives of this study were to elucidate the within-field aphid count variance per sample universe and define within-plant distribution patterns of sugarcane aphid in commercial grain sorghum. The importance of achieving these objectives is to obtain information necessary to design sampling protocols that effectively detect, estimate abundance, and monitor this pest.

In order to accomplish these objectives, a "Russian-doll" approach was used (Chong and Graham 2013, Rimmer et al. 2018) to examine *M. sacchari*'s within-field distributions and finally evaluating aphid distribution patterns within an individual plant's canopy. Two hypotheses were tested: H_1: sugarcane aphid distribution patterns within the sample universe are different based on geographic location and H_2: whole plant enumeration could determine where within the plant canopy the total aphid intensity per plant could best be predicted. By understanding the sugarcane aphid's distribution patterns at each level, sampling plans can be constructed that are more predictive and lead to more accurate and efficient management decisions.

Materials and Methods

Systematic Sampling Protocol

Data were collected from commercial sorghum fields in Kansas, Oklahoma, Texas, and Arkansas during the 2016 and 2017 growing seasons. To qualify as an eligible sampling location, the field had to be a commercial sorghum field measuring at least 16.3 ha. Every sampling event was labeled as a "Field" and analyzed independently, even if the same field was sampled multiple times during the growing season. Growth stage was recorded with every sampling event and was placed into one of five categories: vegetative (1), boot to early flower (2), late flower to milk (3), milk to soft dough (4), and hard dough to black layer (5).

Enumerative sampling was conducted using a stratified sampling protocol to identify infestation patterns. To maintain consistency among data sets from multiple states, every collaborator was trained to use the sampling protocol either personally or by video. All samples were collected in a sampling universe that consisted of a 90m x 90m

grid, divided into nine 30m x 30m cells (Ch. 3: Appendix Figure 12). The sampling universe was then referred to as a "field" and individual fields, that were sampled multiple times, were referred to as "locations".

The systematic sampling grid was placed on the edge of a field and sampling began in the first row of grain sorghum uninterrupted by excessive weeds, or cross-hatch planting, commonly referred to as the first true row. Each cell contained two randomly selected stops in which two leaves on three consecutive plants where examined, totaling six plant samples per cell. The sample unit per plant consisted of two 90% green leaves; one leaf pulled from the upper and one leaf from the lower part of the canopy. Aphids were completely enumerated on each of the two leaves per plant and recorded separately. In addition to the 54 two-leaf plant samples per field, six of the sampled plants were randomly selected for whole-plant enumeration. Aphids were enumerated on each leaf, with at least 90% green leaf area, on the entire plant. Counts began with the lowermost leaf and concluded with either the upper most leaf or the flag leaf if present. Each leaf was labeled beginning with the bottom leaf as number one, the second leaf as number two, and continuing until the last leaf as number *x*.

Statistical Analysis

The first analysis conducted but not discussed heavily till chapter four were regression analyses performed to determine the dispersion pattern of *M. sacchari*. Using the method described in chapter four, Taylor's power law revealed two sampling regions based on significantly different *b* values: a northern region consisting of north Texas, Oklahoma, and Kansas, and a southern region consisting of south Texas and Arkansas. In

an earlier study, Elliott et al. (2017) indicated that there might be two separate aggregation factors based on geography. The evidence provided in these two studies led to the use of regions in addition to the individual states for the rest of this study.

A nested analysis of variance (NANOVA) was conducted using PROC NESTED (SAS 9.4). The data were classified into independent variables by state, by individual field, by column number within the sampling grid, cell number within the column, stop number and plant number within the cell. The dependent variable was total number of *M. sacchari* per plant. All states were analyzed individually, within designated regions, and as one data set.

Assessments of within-plant canopy distribution of *M. sacchari* were also characterized using a modified technique described by Lampert (1989). Leaves from every plant were assigned into a canopy category of upper (3), middle (2), and lower (1) position. The upper two leaves having 90% or more green surface, including the flag leaf, was assigned into the upper canopy. The lowest two leaves having 90% or more green surface area were assigned into category 1, and any leaf with at least 90% green surface area that fell between categories 1 and 3 were assigned into category 2. Because there were variable numbers of leaves assigned into category 2 (due to plant size and growth stage) growth stage was recorded for each sampling event. Lastly the data were categorized by state and region.

PROC REG (SAS 9.4) was used to run regression analyses for model fitness regressing canopy position to total *M. sacchari* per plant. Similar models were analyzed using multiple independent variables like canopy position and state, canopy position and

growth-stage, and canopy position, growth-stage, and state combined vs. total *M. sacchari* per plant. In addition to the standard regression relationships a fixed effects model was used to evaluate the relationship between canopy position and total number of *M. sacchari* per plant within growth stages and states. This model was performed by region.

Results and Discussion

Stratified Sampling Protocol

From 2016 to 2017, complete enumeration of *M. sacchari* was completed on 15,174 two-leaf samples and 1,644 whole plant counts from 281 sampling events (fields) at 134 different locations across Kansas, Oklahoma, Texas and Arkansas (Table 1). All five growth stages were represented across all four states.

Table 1: Summary of sampling data from all states and years 2016 and 2017

	Sampling Locations			**Fields**			**Number of Whole Plant Counts**		
State	2016	2017	**Total**	2016	2017	**Total**	2016	2017	**Total**
Kansas	7	10	**17**	7	10	**17**	0	60	**60**
Oklahoma	25	28	**53**	59	81	**140**	354	486	**840**
North Texas	12	5	**17**	51	17	**68**	306	102	**408**
South Texas	14	19	**33**	17	19	**36**	102	114	**216**
Arkansas	8	6	**13**	14	6	**20**	84	36	**120**
Total	**66**	**68**	**134**	**148**	**133**	**281**	**846**	**798**	**1644**

Sampling summary including locations, sampling events (Fields), and number of whole plant counts for both 2016 and 2017 years.

Nested Analysis of Variance

The nested analysis of variance (NANOVA) was used to determine where, within the sampling universe, does most of the sampling count variance occur. Understanding and accounting for the amongst counts variance provides critical insight into best means of sampling. The (NANOVA) was analyzed by combining sampling events by region (Table 2). Within the northern region, over 73% of the total variance within the sampling universe was accounted for by sampling the upper and lower leaves of three plants in a row. An additional 12% of the variance was attributed to the two samples of three within a 30X30 meter cell. As a result, just by sampling three consecutive plants in two stops within a cell, over 85% of the total aphid count variance being accounted for. Zero variance was seen amongst counts in cells or columns. An additional 12% variance was accounted for when each field and state is sampled independently bringing the total variance accounted for up to 97%. To summarize, in the northern region, most count variance can be attributed to sampling two sets of three plants in a row within a 30m radius per field. This gives rise to the concept that *M. sacchari's* distribution within a field is so highly clumped that the largest amongst sample count differences in this region are amongst the sorghum plants themselves. Furthermore, this finding may suggest that sampling a field in one area may be enough, even if the field is larger than 16.2 hectares.

Table 2: Nested analysis of variance (NANOVA) results by region

Comparison	NR[a]: Variance Accounted for in %	SR[b]: Variance Accounted for in %
Amongst States	**2.69**	**0.00**
Amongst Fields	**11.82**	**27.65**
Amongst Columns	**0.00**	**7.81**
Amongst Cells	**0.00**	**9.35**
Between Samples	**12.11**	**35.09**
Amongst Plants	**73.38**	**20.11**
Total Variance	**100**	**100**

Demonstrates percent of variance accounted for by strata. Northern region, consisting of Kansas, Oklahoma, and North Texas=NR[a]; Southern region, consisting of South Texas and Arkansas=SR[b].

Within the southern region the highest amount of sample variance was accounted for by the two samples of three consecutive plants within a 30m radius. Sampling in this manner resulted in over 55% of the aphid count variance being accounted for. Unlike the northern region there was approximately 17% variance accounted for amongst cells and columns indicating that where the samples are taken in relation to an edge of the field is an important factor to consider. Lastly sampling fields independently resulted in an additional 28% total variance amongst fields. Based on the NANOVA analysis, samplers located the southern region should sample each field independently, sample two stops of three plants in a row, and cover at least 90m of distance within a field beginning at an edge and working inward.

The nested analysis allows for deduction of how to effectively account for sampling variance within a given sampling universe. The conclusion from this analysis is that samplers in both regions benefit from taking two samples of three plants in a row within a 30m proximity of one another per field. To account for more variance in the southern region, being sure to sample beginning at an edge and moving into the field can

only increase precision. By following these guidelines, the chances for early detection and more precise monitoring can be improved.

Within Canopy Distribution

The within canopy relationships were further clarified by analyzing whole plant enumeration samples. In order to make all plant samples relatable across multiple grain sorghum varieties with varying numbers of leaves, the plant canopy was partitioned into three categories: upper, middle, and lower. The "upper" and "lower positions" were defined as the two 90% green upper and lower most leaves. Anything between the two upper-most and two lower-most leaves was classified as the "middle" canopy position. All plants with only one middle position leaf were excluded from the data set leaving a total of 790 plants in the analyses.

In the first analysis, the upper, middle, or lower canopy position was regressed against the *M. sacchari* total per plant (Table 3). This analysis did not consider region, state, or growth stage. The results of this analysis showed that best fit model was the mid canopy position with an r^2 of 0.9131. The lowest relationship to the total plant density belonged to the lower canopy position which had a r^2 of 0.6554. This analysis demonstrates that overall, without accounting for the region, state, or growth stage of the sorghum, the total data trend reveals that the middle of the plant canopy across all states, growth stages, and both regions is the most predictive of the total plant aphid intensity.

Table 3: Within canopy distribution regression analysis results

Position	Number of Plants	MSE	F-Statistic/p-value	R^2
Upper	790	7.304	2201.48/<0.0001	0.7364
Middle	**790**	**4.195**	**8276.38/<0.0001**	**0.9131**
Lower	790	8.351	1498.65/<0.0001	0.6554

Relationships between the within canopy position of *M. sacchari* colonies and the total number of *M. sacchari* on the entire plant regardless of state and growth stage. All values with p= <0.05 are considered significant. Best fit models, determined by r^2 value, are shown in bold.

The second analysis evaluated which region of the plant canopy is more predictive of the total plant aphid intensity by state. The results of this analysis showed, again, that across all states, despite the growth stage, the middle position of the plant canopy is most predictive of the total plant *M. saccharii* density (Table 4). Similarly, when the variable of state is removed, looking at all five growth stages, the middle position of the plant canopy continues to be the most predictive of the total plant density by r^2 value (Table 5). The multiple regression model including state, growth stage, and canopy position as predictors of the total *M. sacchari* density per plant showed that out of the 50 significant relationships, 22% of the time the middle was most predictive followed by the upper canopy at 10% and the lower 8% (Ch. 3: Appendix Table 12).

Table 4: Within canopy distribution regression analysis incorporating state

Region	State	Position	Number of Plants	MSE	F-Statistic/p-value	R^2
Northern	Kansas	Upper	25	1.474	2.65/0.1171	0.1034
		Middle	**25**	**1.051**	**27.37/<0.0001**	**0.5434**
		Lower	25	1.455	3.32/0.0815	0.1261
Northern	Oklahoma	Upper	243	6.035	705.28/<0.0001	0.7453
		Middle	**243**	**3.870**	**2271.36/<0.0001**	**0.9041**
		Lower	243	9.447	180.46/<0.0001	0.4282
Northern	North	Upper	222	4.101	367.42/<0.0001	0.6255
	Texas	Middle	222	3.118	796.15/<0.0001	0.7835
		Lower	**222**	**2.180**	**1857.96/<0.0001**	**0.8941**
Southern	South	Upper	125	8.379	484.20/<0.0001	0.7974
	Texas	**Middle**	**125**	**5.516**	**1278.20/<0.0001**	**0.9122**
		Lower	125	10.206	286.30/<0.0001	0.6995
Southern	Arkansas	Upper	93	12.810	145.43/<0.0001	0.6151
		Middle	**93**	**5.158**	**1366.97/<0.0001**	**0.9376**
		Lower	93	11.281	213.83/<0.0001	0.7015

Relationships between the within canopy position of *M. sacchari* colonies and the total number of *M. sacchari* on the entire plant separated by state, regardless of growth stage. All values with p= <0.05 are considered significant. Best fit models, determined by r^2 value, are shown in bold.

Table 5: Within canopy distribution analysis incorporating state and growth stage

Growth Stage	Canopy Position	Number of Plants	MSE	F-Statistic/p-value	R^2
Vegetative	Upper	90	10.403	333.25/<0.0001	0.7911
	Middle	**90**	**4.345**	**2326.77/<0.0001**	**0.9636**
	Lower	90	10.255	345.48/<0.0001	0.7970
Boot to <50% flower	Upper	116	2.754	440.75/<0.0001	0.7945
	Middle	**116**	**2.473**	**573.68/<0.0001**	**0.8342**
	Lower	116	3.266	280.21/<0.0001	0.7108
>50% flower to Milk	Upper	207	7.463	554.38/<0.0001	0.7300
	Middle	**207**	**2.885**	**4875.82/<0.0001**	**0.9597**
	Lower	207	7.391	569.21/<0.0001	0.7352
Milk to Soft Dough	Upper	143	6.058	238.80/<0.0001	0.6288
	Middle	**143**	**4.116**	**681.81/<0.0001**	**0.8286**
	Lower	143	5.108	393.18/<0.0001	0.7360
Hard Dough to Black Layer	Upper	243	7.234	654.78/<0.0001	0.7384
	Middle	**243**	**2.448**	**1331.21/<0.0001**	**0.8516**
	Lower	243	10.893	159.07/<0.0001	0.4068

Relationships between the within canopy position of *M. sacchari* colonies and the total number of *M. sacchari* on the entire plant separated by state and growth stage. All values with p= <0.05 are considered significant. Best fit models, determined by r^2 value, are shown in bold.

Overall, based on these analyses the middle position of the plant canopy is the most predictive position within the plant canopy regardless of region, state, and growth stage when all were evaluated independently. Although this general trend of middle position importance, continued when all the predictor variables were included, there were

few significantly different scenarios. Some states, at certain growth stages showed a different canopy position to be most predictive (Ch. 3: Appendix Table 1).

It is important to consider that these models could be biased towards the middle position canopy since only two upper-most and lower-most leaves were considered for the other two positions, making many of the leaves in some growth stages the middle. Plants with only five leaves were removed from the analysis so every position had a minimum of two leaf representation. The maximum number of leaves on a plant was 12 with an average of 9.5 leaves at growth stages 2-4, which had the most 90% green leaves. The correlation issue in this initial model is more than likely amplifying a trend that may not be as strong as this data suggests. This fundamental issue will lead to future models that will remove bias by either randomly selecting two leaves from the middle of the canopy or combing the upper and lower canopy positions.

Even with correlation bias due to more leaves existing in the middle of the canopy, these results are still very important for practicality since the accepted manner of sampling for sugarcane aphid is to take an upper most and lower most leaf. These data suggests that this arbitrary two leaf sampling unit, though meant to be representative of the whole canopy distribution, may not be as representative as once presumed, and could be leading to a misinterpretation of the population intensity present. The inaccurate sampling unit being used may contribute to the idea that this aphid's population "blows-up" when really sampling has just not been representative of the true intensity per plant. Moving forward, perhaps a combination of two middle leaves, an upper and a middle, or any other combination including a middle leaf may be more representative of the intensity per plant.

Conclusions

This study allowed for understanding of the within field colonization patterns of the grain sorghum pest *M. sacchari*. Better understanding of how this pest colonizes a field can lead to a research-based approach at sampling to determine population intensity. Using the statistical and empirical models in this study, suggested that sugarcane aphid aggregation patterns vary by field and geographic location, and could influence the constructs of effective sampling plans that are developed for *M. sacchari*. In our study, aggregation patterns were different in fields located in Kansas, Oklahoma, and northern Texas than fields from Arkansas and South Texas. This study found that the best means of sampling a field regardless of region is to take two samples of three plants in a row within a 30m radius. If in the southern region it is beneficial to begin at a field edge and working into the center. This study indicated that while there are few scenarios that are statistically significantly different when all variables are considered, the trend is that a middle leaf in the plant canopy may increase predictability as a sample unit. When these results are integrated into a sampling plan at a field level there is no doubt that more effective measurements of intensity will be obtained leading to better management decisions.

CHAPTER IV

ENUMERATIVE SAMPLING PLANS FOR *MELANAPHIS SACCHARI* (ZEHNTNER) IN COMMERCIAL GRAIN SORGHUM: FIXED SAMPLE SIZE AND SEQUENTIAL SAMPLING PLANS FOR POPULATION ESTIMATION AND CLASSIFICATION

Abstract

Sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) has been an economic pest of grain sorghum in the United States since 2013. Integrated pest management techniques, including host plant resistance, planting dates, and insecticide treatments have been developed to effectively manage the pest. Presumably, the capacity to determine effectiveness and reliability of these tools depends on a researcher's ability to monitor sugarcane aphid populations in the field. Yet, no research-based sampling plans have been developed to reliably estimate or classify sugarcane aphid intensities. The objective of this study was to develop three common enumerative sampling plans for sugarcane aphid in grain sorghum and evaluate their in-field practicality. Using 134 grain sorghum fields from four states, Taylor's power law regressions identified two clumped, but significantly different aggregation factors based on geographic location.

The aggregation patterns identified in Kansas, Oklahoma, and northern Texas (identified as northern sampling regions) were like each other but different from patterns recorded in southern Texas and Arkansas (identified as southern sampling regions. Enumerative and sequential sampling plans using fixed precision levels of 0.10 and 0.25 for three action levels (low, medium and high) were developed and evaluated for each region. A fixed sample size curve was also developed. Sequential sampling models for population density estimation and population intensity classification using a cumulative number of aphids vs. the number of plants sampled method were developed. With one exception, the number of samples required to monitor this aphid with an acceptable level of precision far exceeded a realistic number for use in an applied sampling construct. This study provides evidence for the need of a binomial sequential sampling plan that quickly and efficiently classifies these populations at critical densities.

Introduction

Melanaphis sacchari (Zehntner), (Hemiptera: Aphididae) commonly referred to as the sugarcane aphid, has been a moderate to severe economic pest of commercial grain sorghum since 2014 (Bowling et al. 2016). Since the first report of *M. sacchari's* damage to grain sorghum in 2013, a substantial effort has been put forth to develop sustainable integrated pest management techniques for sugarcane aphid. While host plant resistance and insecticides are able to gain control of the pest at identified regional thresholds (Brewer et al. 2019), there is still no effective way to determine the density of a population, or classify a population's intensity relative to a treatment threshold.

Before developing a sampling plan, it is critical to define the population's statistical dispersion pattern (Pedigo and Buntin, 1994). There are several different

formulas for accomplishing this, one of the most widely accepted methods is to use the empirical model called Taylor's power law (Taylor 1961). Taylor's power law (TPL) is a robust empirical model for defining mean to variance relationships that often relate to dispersion pattern. TPL does not rely solely on the mean of a sample universe to define the population, therefore, it is less variable over time and space (Pedigo and Buntin, 1994). TPL assigns a slope value (b) to the linear relationship between the mean and variance of multiple sampling universes (Elliott 1977). This allows for a more reliable measure of spatial distribution over a multi-dimensional environment.

Since the mid-19th century, enumerative sampling, whether by a fixed sample size or sequential sampling for population estimation/classification, has been used by scientists to estimate and monitor insect populations (Moon and Wilson, 2009; Pedigo and Buntin, 1994). There are two main reasons for sampling insects: to determine a density within a given environment or to classify a population below, at, or above a critical density (Pedigo and Rice, 2006). Scientists interested in monitoring important insect population densities for research typically require a higher precision level, usually around 0.10 (Moon and Wilson, 2009). Although less common than sequential sampling for population classification, sequential sampling for estimation of a population density is used in some species depending on the biology and abundance in the environment (Pedigo and Rice, 2006).

On the contrary, for pest management decisions population density or population classification estimations can be set at a lower precision level, usually around 0.25, for practicality because lowering precision often lowers the sample size as well. For monitoring efficiency, sequential sampling plans are usually preferred over fixed sample

size plans because the required minimum sample size is adaptable based on population intensity within the sampling universe. Therefore, sequential sampling plans usually require less samples to provide enough information to make an action decision especially in sampling universes where the intensity is well above or below the critical density (Norris et al. 2003). The common critical density used in integrated pest management is an economic or action threshold based on an economic injury level (Moon and Wilson, 2009).

Elliott et al. (2017) presented preliminary research on sugarcane aphid distribution in part of its geographic distribution range. Yet, sugarcane aphids overwinter in some areas and then migrate extensively over a wider geographic expanse each year. This led to the idea that a more robust data set was needed to accurately characterize its spatial distribution in order to effectively monitor this aphid. The objective of this study was to develop the three most common enumerative sampling plans, (fixed sample size, sequential sampling for population density prediction, and sequential sampling for population classification) for *M. sacchari* in grain sorghum. The first step of this study was to define the aggregation factor or factors of *M. sacchari*. The study ended with developing sequential sampling stop-lines for classification of this aphid at multiple economic thresholds.

Materials and Methods

Systematic Sampling Protocol

For this study the 134 sampling events were conducted in commercial grain sorghum fields measuring at least 16.2 hectares in size in Kansas, Oklahoma, Texas, and Arkansas using the stratified sampling protocol, described in depth in chapter 2. Each

sampling event consisted of aphid counts collected *in situ* from 54 two leaf samples per plant, within a 90m x 90m gridded sampling universe. In addition to the 134 sampling events previously mentioned, 50 additional external sampling events, collected in a similar fashion, were taken from sorghum fields in Kansas, Oklahoma, Texas, Louisiana, and Mississippi and used to validate the sequential sampling for population estimation models.

Defining Dispersion Pattern and Aggregation Factors

Taylor's (1961) empirical model ($s^2 = am^b$) was used to estimate *M. sacchari*'s aggregation in sorghum fields using PROC REG in SAS 9.4 (SAS Institute 2013). In the equation, s^2 = the variance, m= the mean density and *a* and *b* are constants. The intercept (*a*) and slope (*b* values) from TPL are interpreted as a sampling factor and index of aggregation respectively. The index of aggregation (*b*) determines the aggregation type ranging from highly clumped (*b*<1) to randomly distributed (*b*=1) (Taylor, 1961). TPL regressions were calculated for each state, within designated regions, and combined into one overall data set utilizing

$$\boldsymbol{\log (S^2) = \log a + b \log (\bar{x})} \qquad \textbf{(1)}$$

TPL aggregation factors were compared by state using equation 2 for the standard z-test slope comparison:

$$\boldsymbol{\left(Z = \frac{b_1 - b_2}{\sqrt{SEb_1^2 + SEb_2^2}}\right)} \qquad \textbf{(2)}$$

Where *b* is the TPL slope and SEb is the standard error of these slopes. The regional comparisons were also tested using a dummy variable regression conducted by using PROC REG in SAS 9.4.

Fixed Minimum Sample Size Estimation at a Fixed Precision

Green's (1970) formula (3) was used to define the minimum number of samples needed to estimate a population at a disclosed mean achieving a certain level of precision (Green, 1970).

$$\boldsymbol{n = \frac{am^{b-2}}{c^2}} \qquad \textbf{(3)}$$

Where *n* is the sample size, *m* is the sampling mean, *a* and *b* are TPL parameters, and c^2 are the fixed precision levels of 0.10 and 0.25. For this estimation, the means used in the formula were proposed economic thresholds per plant using a two-leaf sample (Gordy et al. 2019). To build minimum sample size curves with fixed precision, the mean in equation 3 was replaced with *n* = number of aphids per sample resulting in a relationship between the number of samples required for *n* number of aphids per sample.

Sequential Sampling for Population Estimation

Predicted stop-lines for fixed precision population estimation were generated using equation 3 described by Green's (1970) formula at precision levels of 0.10 and 0.25.

$$\boldsymbol{logT_n = \frac{\ln\left(\frac{D^2}{a}\right)}{b-2} + \frac{b-1}{b-2} ln} \qquad \textbf{(4)}$$

Where T_n = the cumulative number of aphids sampled, n = the total number of samples, D^2 = desired precision level, and *a* and *b* = TPL parameters mentioned above. Stop-lines using Green's fixed-precision sequential sampling formula were validated using Resampling for Validation of Sampling Plans (RVSP) developed by Naranjo and Hutchison (1997). In total 50 previously mentioned externally sampled fields were used to validate the sequential sampling for population estimation developed by equation 4. Each field was "sampled" with replacement over 500 iterations from which average sample sizes and average precision levels based on the two desired precision levels were calculated. Using the RVSP data output per field, min, mean, and max sample size and precision curves were developed spanning a range of sample sizes.

Aphid Population Classification by Sequential Sampling

Unlike sequential sampling for population estimation, sequential sampling for classification is used to determine if (yes or no) the population intensity is above a critical density. For insects classified as pests like *M. sacchari*, this critical density is typically an economic threshold (ET). The stop-lines generated for this purpose are used to classify the pest population as above or below an ET in order to make a treatment decision to prevent intensities from reaching the economic injury level (EIL) where economic loss occurs. To estimate classification, stop-lines for negative binomial populations equations 6 & 7 developed by Wald (1947) were used. A negative binomial distribution was assumed due to the populations being highly aggregated, but not confirmed. Before stop-lines could be developed, Wald's stop-lines require a common *k* to be calculated. A TPL adaption of the common *k* equation (Wilson and Room, 1983) was used to develop an

overall common *k* by generating a *k* value per sampling event then taking a total average. The adapted *k* formula (equation 5) is listed below.

$$\boldsymbol{k} = \frac{\boldsymbol{m}}{\left(\boldsymbol{am^{(b-1)}-1}\right)} \qquad \textbf{(5)}$$

Where *m* = the data mean calculated in the typical manner, and *a* and *b* are TPL parameters mentioned above.

$$\mathbf{d_1 = (b*n) + h_1} \qquad \mathbf{d_2 = (b*n) + h_2} \qquad \textbf{(6)}$$

$$\mathbf{b} = \boldsymbol{k} * \left(\frac{ln\left(\frac{q_2}{q_1}\right)}{ln\left(\frac{p_2 * q_1}{p_1 * q_2}\right)}\right) \qquad \mathbf{h_1} = \left(\frac{ln\left(\frac{\beta}{1-\alpha}\right)}{ln\left(\frac{p_2 * q_1}{p_1 * q_2}\right)}\right) \qquad \mathbf{h_2} = \left(\frac{ln\left(\frac{1-\beta}{\alpha}\right)}{ln\left(\frac{p_2 * q_1}{p_1 * q_2}\right)}\right) \qquad \textbf{(7)}$$

Where m_2 and m_1 = the ET and 0.33 of the ET as suggested by published EIL's (Gordy et al., 2019), $p_1 = (m_1/k)$, $p_2 = (m_2/k)$, $q_1 = (1+p_1)$, and $q_2 = (1+p_2)$. Where *k* = common *k*, an aggregation factor calculated in equation 6, and in equation 4, d_1 =the upper stop-line, d_2 = the lower stop-line, and *n* = the number of plant samples.

Results and Discussion

Aggregation Factors

According to Taylor (1961), *b* values that are less than one describe a regular distribution, around one meaning a randomly distributed population, and a *b* greater than one is considered to be aggregated (Pedigo and Buntin, 1994; Taylor, 1961). For this study, data from each state were analyzed individually by regressing the log-mean to log variance (Table 6). The *b* values from each state ranged from 1.82 (Oklahoma) to 1.66 (South Texas and Arkansas). All values were clumped.

Table 6: Taylor's power law regression results by region and state

State/Region	n	Log α± SE	T, p-value	**Log b± SE**	T, p-value	r^2
Northern Region	**207**	**2.58±0.054**	**47.62,<.0001**	**1.76±0.026**	**67.10,<.0001**	**0.96**
Southern Region	**53**	**2.56±0.164**	**15.62,<.0001**	**1.65±0.043**	**38.41,<.0001**	**0.97**
Kansas	17	2.54±0.185	13.74,<.0001	1.72±0.075	22.84,<.0001	0.97
Oklahoma	131	2.82±0.061	46.45,<.0001	1.82±0.030	61.88,<.0001	0.97
North Texas	59	2.13±0.117	18.16,<.0001	1.773±0.060	29.75,<.0001	0.94
South Texas	33	2.66±0.188	14.15,<.0001	1.66±0.051	32.31,<.0001	0.97
Arkansas	20	2.32±0.346	6.72,<.0001	1.66±0.084	19.70,<.0001	0.96

Aggregation factor analysis results using Taylor's Power Law. Categorized by location, number of sampling events (n), alpha and beta coefficients (Log α, Log b) with standard error (SE), T-test values with p-values, and goodness of fit score (r^2). Aggregation factor is determined by the slope (Log b); values lie between 1 and 2: 1 being classified as a random distribution, and 2 being classified as very highly aggregated distribution.

A z-test was performed on the *b*-values from the TPL analyses. Results from the z-test indicated that slopes (*b*-values) derived from fields in Oklahoma, Kansas, and North Texas were not significantly different (z = 0.50527, df= 1 p=0.3085). Likewise, there was no significant difference in *b*-value between south Texas and Arkansas (z = 0.00738, df=1, p > 0.5000). States with no significant differences in slope were combined and re-analyzed (Table 6). The northern region's (Kansas, Oklahoma, and north Texas) combined *b*-value (*b* = 1.76) and the southern region's (south Texas and Arkansas) b value (*b* = 1.65) were significantly different (z= 1.8432, df=1, p=0.0329). To confirm that there was a significant difference in TPL values between the northern and southern region, a dummy variable regression analysis was also performed and confirmed that the two region's aggregation factor were significantly different from one another (t = -1.89, df =1, *p* = 0.0507).

In theory, TPL values are associated with different species in an environment (Pedigo and Buntin 1994). However, it is not entirely uncommon for an aphid species,

being very highly clumped, to have different *b*-values based on environmental conditions. In the Southern Great Plains, Giles et al. (2000) reported different *b*-values for greenbug distribution in winter wheat that were dependent on the time of year (fall vs. spring). In fall following wheat emergence, most greenbugs likely migrate to wheat from other hosts, whereas in spring, greenbugs originate from both overwintered populations in wheat and migrating populations from other hosts.

Elliott et al. (2017) found that *M. sacchari* in Texas sorghum displayed an aggregation factor value that was significantly different from Kansas, Oklahoma, Louisiana, and Arkansas. They suggested that differing TPL values reported potentially related to differing overwintering strategies (Brewer and Gordy, 2016) from southern states/regions to northern states/regions and could result in a need for different sampling protocols for a northern and a southern region. Although these results did not arrive at the same separation by states, they did suggest that there were differences in aggregation patterns based on geography. One explanation for the incongruity between the two studies may be reflected in the number and location of sampling events that this study encompassed relative to the data collected by Elliott et al. (2017). This study included 50 sampling events split between south Texas and northern Texas, while the study reported by Elliott et al. (2017) included 17 sampling events from Texas and did not indicate their locations within the state. Additionally, this study included 2x sampling events from Arkansas collected over two years compared to those that originated from Arkansas by Elliott et al. (2017). The greater number of sampling events collected over two different years may account for the slight divergences in results.

Furthermore, spatial distribution separation between regions may be explained by over wintering patterns. Bowling et al. (2016) provided observational evidence that sugarcane aphid can overwinter on alternate hosts below 32° latitude and indicated that reports of seasonal patterns of detection over several years were consistent with the explanation that *M. sacchari* populations build in overwintering areas of North America and expand northward through wind-aided movement. Another study by Michaud et al. (2018) demonstrated in growth chambers that while *M. sacchari* can have relatively high survival rates at temperatures as low as -4 degrees Celsius, cultivated sorghum as well as *M. sacchari's* common weed host johnsongrass, cannot. Therefore, the ability to overwinter successfully is not necessarily based on the biology of the aphid, but on the survival of a suitable host. Differences in statistical distribution patterns of *M. sacchari* seem to coincide with the presence of a living host all winter long.

Additionally, cultivation differences may aid in differences seen between regions. In the southern region, sugarcane aphid is locally present on surviving johnsongrass when grain sorghum is planted in late March and April. *Melanaphis sacchari* presumably moves from the surrounding johnsongrass, using short directed flights into the vegetative-stage grain sorghum. This type of local movement into the field from the edge could be what drives the less clumped aggregation factor seen in the *b*-value from the southern region.

In contrast, *M. sacchari* has been reported to arrive, at the earliest, in late June to early July in the northern region when early planted sorghum is already at late vegetative or early flowering growth stages (Backoulou et al. 2018). Unlike the southern region, there are no living hosts available for the aphid to survive by the time cultivated sorghum

is a viable host. Therefore, *M. sacchari* in the northern region is deposited randomly in the environment as a result of long undirected flights where the aphid was swept up in long distance wind patterns (Brewer et al. 2019). The result of these undirected depositions are small colonies building in pockets throughout the field rather than building from the edge. These differences in deposition and colonization could account for the differences in clumping within the field.

(Parry, 2013) outlined four "basic rules" in the areal transport process for cereal aphids. They include: (1) uplift at the source, (2) transportation in the atmosphere, (3) deposition leading to initial distribution following transportation, and (4) subsequent local movement. Wallin and Loonan (1971) summarized observation patterns of dispersal for the aphid vectors of barley yellow dwarf virus via jet-streams in North America (Wallin and Loonan 1971); however, little is known about the local and long-distance migration patterns for sugarcane aphid in North America. Other studies have shown highly clumped, low intensity populations increase variability of TPL *b* values for the same species (Lepš 1993). Yet, sampling for this study was intentionally conducted during the early stages of sugarcane aphid colonization when population intensities were at, or below the current recommended treatment thresholds to ensure a functional sampling protocol. To remedy the possibility of high variance in the TPL values, samples that represented wide-ranging population intensities (0.7 to 550 aphids per leaf) were included in all data sets. This robust data set leads to the conclusion that the aggregation differences reported herein between the southern and northern regions are robust.

Minimum Sample Sizes at a Fixed Precision

Minimum sample sizes at fixed precisions were calculated for a high, medium, and low economic thresholds (ET) based on recent data which reports the economic injury level and corresponding ET's per leaf, for fast and slow growing populations, across a similar geographic region (Gordy et al. 2019). All models developed for this study were based on a per plant average of aphids collected from a two-leaf sample. Gordy et al. (2019) reported ETs based on a per leaf average; therefore, we doubled the ETs reported by Gordy et al. (2019) to develop the models. Minimum samples sizes for the northern region were reported for ET's of 200, 150, and 75 *M. sacchari* per plant based on a two-leaf sample at both 0.10 and 0.25 precision levels (Table 7).

Table 7: Minimum Sample Size in northern and southern region at high, moderate, and low economic thresholds at precision levels of 0.25 and 0.10

	Fixed Precision Level					
	Per Plant ET High (NR=200,SR=100)		Per Plant ET Moderate (NR=150,SR=80)		Per Plant ET Low (NR=75, SR=50)	
Region	0.25	0.10	0.25	.10	0.25	0.10
Northern	60	374	64	400	76	472
Southern	41	258	45	279	53	329

Using Green's (1970) formula, minimum sample size values are reported at fixed precision levels. NR= Northern region, SR= Southern Region, and ET= Economic threshold.

Likewise, minimum sample sizes for the southern region were reported for ET's of 100, 80, and 50. The minimum sample size required to estimate aphid intensities for both regions at a fixed precision of 0.10 ranged from 258 to 472 plant samples. The minimum sample sizes required for a fixed precision of 0.25 ranged from 41 to 53 in the

southern region and 60 to 76 for the northern region (Table 7). Over the range of ETs, the northern region required 21 to 23 more samples at a fixed precision of 0.25 and 116-143 more plant samples at a fixed precision of 0.10 compared to the southern region (Table 7)

In addition to minimum samples sizes at fixed precisions calculated for specific ETs, a fixed sample size curve was developed over a range of per plant averages (Figure 1). At extremely low intensities of aphids per plant, the sample size necessary at a fixed precision of 0.10 is well over 1000 samples in both regions. At the lower precision of 0.25, the number of required samples at low intensities exceeded 200 plant samples for both the northern and the southern region. Similarly, to what was reported in Table 2, the northern region requires more samples than are required for the southern region to achieve the same mean and precision level (Figure 1).

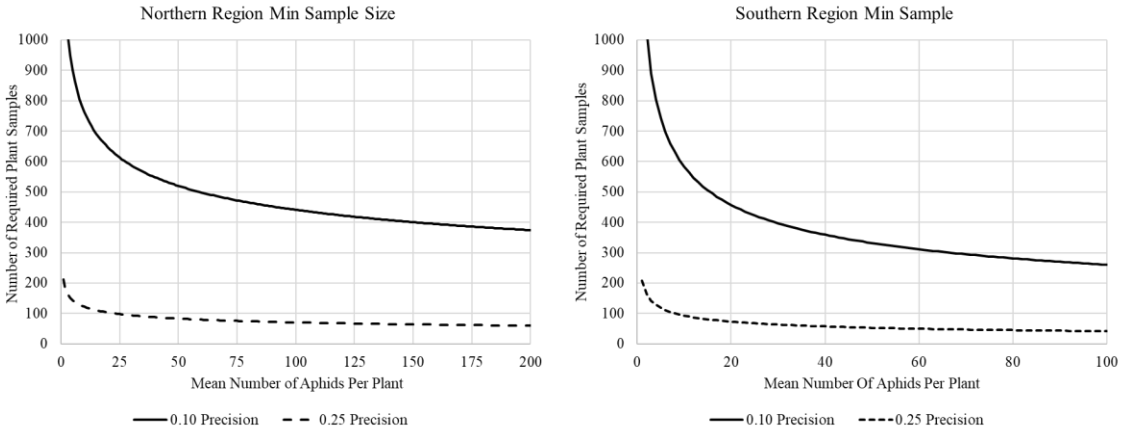


Figure 1: Fixed sample size curves for both regions at 0.10 and 0.25 precision

It is not surprising that the northern region required more samples because a significantly higher degree of clumping occurs in the northern region compared to the southern. Yet, at very low intensities (25 *M. sacchari* per plant or less), the northern and southern region seem to be more similar than in higher intensity populations. This is also

reflected in the TPL *a* intercept being very similar: 2.58 for the northern region, and 2.56 for the southern. The low intensity similarities could be due to two things; very low intensities have higher levels of unavoidable sampling error (Elliott et al. 2003), or the southern region may have more representatives of the "fast" development rate populations as described by Gordy et al. (2019) resulting in larger differences at higher intensities. Overall, at all treatable intensities, these minimum samples size models suggest that in the northern region substantially more samples than in the southern region are required to meet the same level of fixed precision.

Sequential Sampling for Population Estimation

Using Green's (1970) equation 3, fixed precision stop-lines were calculated to estimate population density for both the northern and southern regions (Figure 2). Like the trend observed with the fixed sample size model, more samples were required to reach a stop-line for the northern region at both tested precision levels. In the northern region, a sampler would need a cumulative aphid count >1000 to reach a stop line from 20 plant samples at a 0.25 precision level. In contrast, a sampler would reach the first stop line, at a precision level of 0.25, when the cumulative aphid count reached just under 300 from the same number of plant samples. In both the northern and the southern region, the 0.10 precision level stop line was substantially higher than the 0.25 precision stop-line. This model suggests, again, that at very low *M. sacchari* densities the sampler would need to take well over 80 or 100 samples to obtain a high level of precision. The high number of samples needed to reach a stop line for a precision level of 0.10 in aphids is not unprecedented for aphids being a relatively small insect and having a negative binomial distribution (Elliott et al. 2003).

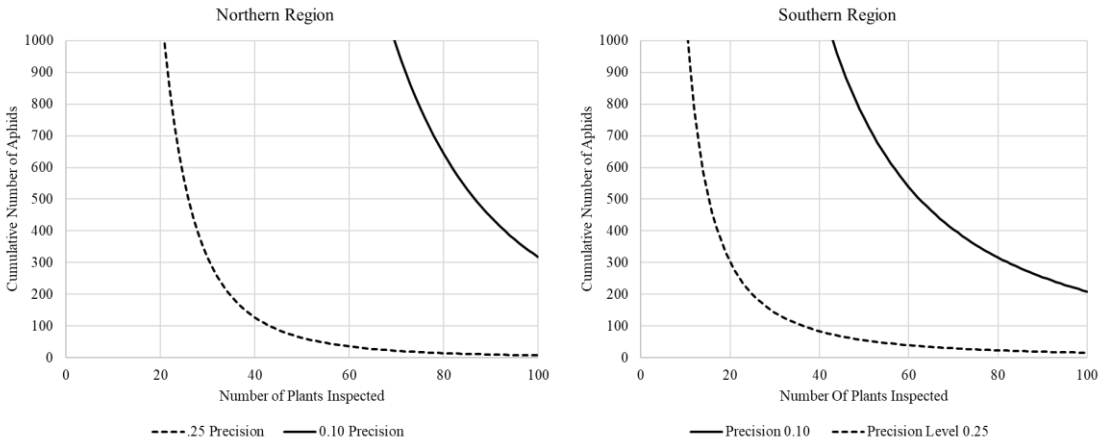


Figure 2: Green's (1970) fixed precision sequential sampling stop-lines for population density estimation

Average validation results of this model (Table 8) suggested that many more plant samples could be required to estimate the population density than the model initially suggested. The northern and the southern region would require sample sizes in the hundreds to achieve either level of precision. Fitting the model better than the average sample size, the validation model predicted average precision for each region was always below the desired precision.

Table 8: Resampling for validation of sampling plans (RVSP) average data output over 500 iterations

				Average Predictions for Fixed Precision-Sequential Sampling Plan Across 500 Iterations					
				Precision (D)			Average sample Size		
Region	N	Desired Precision	Observed mean Intensity (per plant)	Mean	Min.	Max	Mean	Min.	Max
Northern	25	0.1	58.5	0.09	0.08	0.1	914	200	978
Northern	25	0.25	58.5	0.22	0.15	0.3	147	124	178
Southern	25	0.1	142.5	0.1	0.08	0.11	582	190	642
Southern	25	0.25	142.5	0.23	0.13	0.31	95	76	121

Average estimations for precision and average sample size based on 25 independent validation data sets per region. Validation sampling iterations based on Green's (1970) formula for sequential sampling for population estimation. Desired precision was 0.10 or 0.25 and minimum sample size was set to 10.

The minimum, mean, and maximum sample size prediction and precision curves (Figures 3 and 4) align with the calculated fixed-precision model better than the total averages. These figures represent the more realistic range of data across multiple population densities. A reason there could be slight discrepancy between the stop-lines estimated by Green's and the average validation output was the use of the "with replacement" option when running the 500 sampling iterations. Used commonly (Naranjo and Castle 2010, Naranjo and Hutchison 1997, Tran and Koch 2017), the replacement option skews the field data slightly in order for the iteration to continue in highly clumped populations with many zeros.

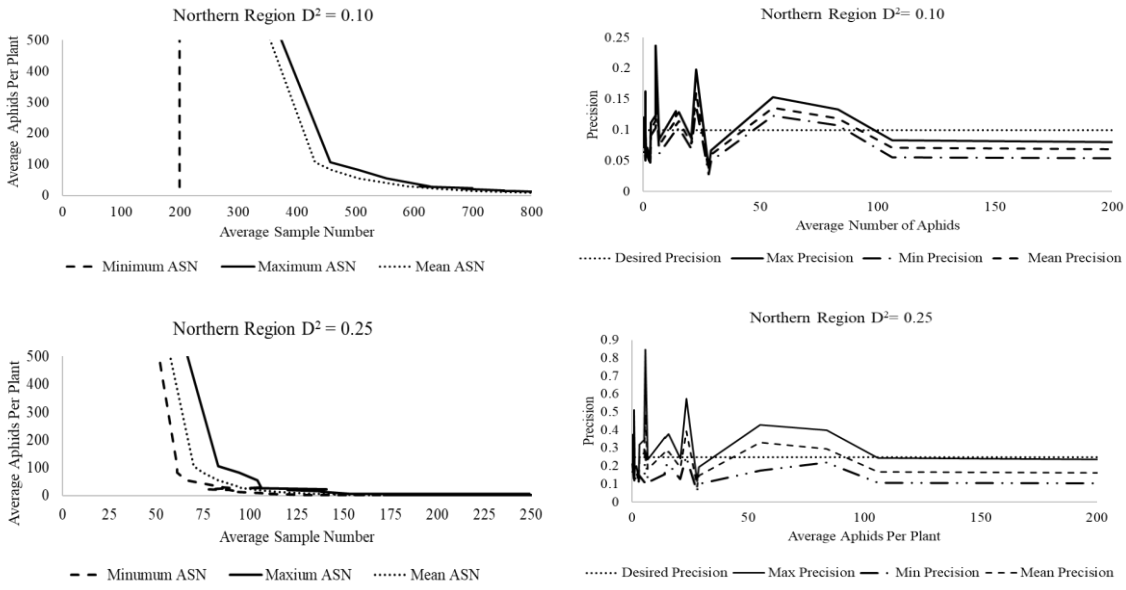


Figure 3: Northern region average sample number and precision curves from RVSP output

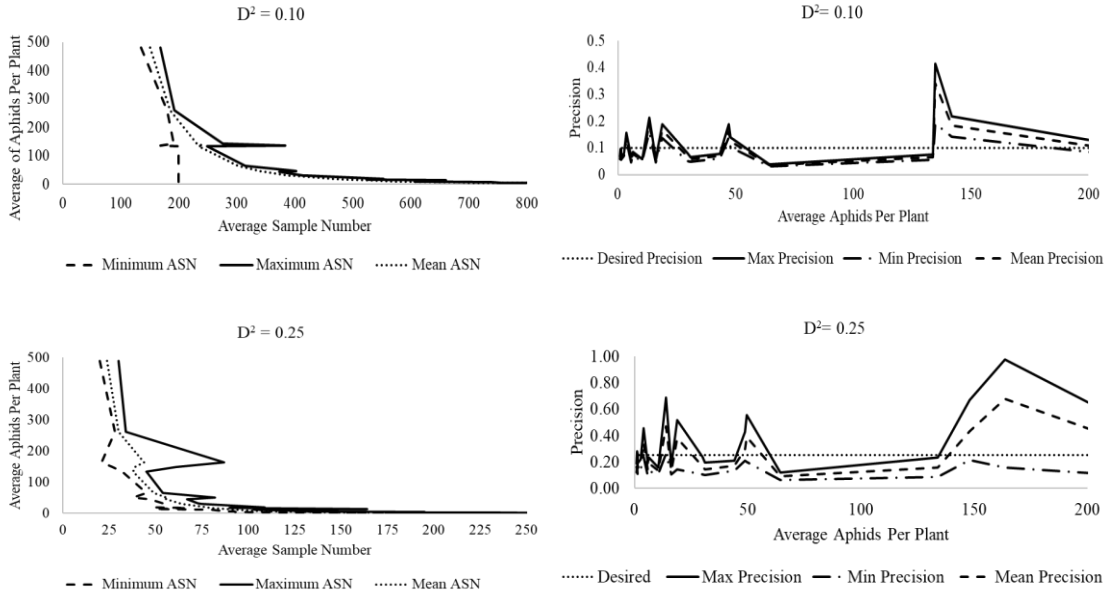


Figure 4: Southern region average sample number and precision curves from RVSP output

In summation, the validation results seemed to confirm the results of the model calculated using Green's formula to estimate population density at fixed precision levels. As for application of this sequential sampling model for population estimation, only in the southern region could this model be deemed efficient due to lower sample sizes. Even so, having to count hundreds of aphids before reaching a stop-line may take too much time for most producers and lead to estimates that introduce a large amount of sampling error. In the northern region enumerative sequential sampling to estimate population density due to high sample sizes and high cumulative aphid counts, seems unreasonable for an applied sampling protocol.

Wald's (1947) Sequential Probability Ratio Test (SPRT) Stop-Lines

The common *k* derived from equation 4 was used to describe aggregation of insects was calculated for the northern region (0.579057) and southern region (1.888831) was more incongruent than TPL previously suggested. The stop-lines show that at 5% and 10% alpha and beta error in both regions the minimum sample size to cross and upper or a lower stop-line would be at 20 plant samples (Figures 5 and 6). To be expected the highest threshold (200 aphids per plant) requires the most cumulative aphids to reach a treatment threshold. However, true to the other two sampling plans described in this paper, fields in the southern region require much lower cumulative aphids to reach a treatment threshold than those in the northern region regardless of ET. At the high ET of 100, the southern region only requires 1000 cumulative to reach a treatment threshold within 20 plant samples. In comparison at an ET of 75 aphids per plant, the northern region requires 2300 or more aphids to reach a treatment threshold in the same 20 plant

samples. This variation in cumulative aphid requirements can be attributed to the significant differences in aggregation seen in both the TPL and common *k* values.

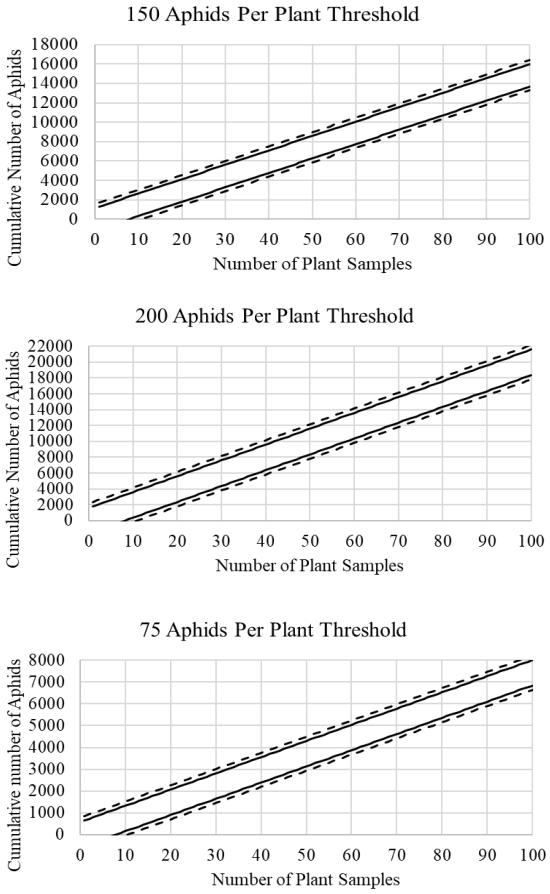


Figure 5: Wald's (1947) SPRT stop-lines for classification of *M. sacchari* at treatable and non-treatable levels for the northern region. The upper and lower limits of the stop-lines are dashed at 5% and solid at 10% alpha and beta error. If cumulative aphids at a given number of plant samples is above the upper limit insecticidal treatment is warranted at that threshold. If the minimum sample size of 20 plants had less than the lower limit of cumulative aphids, no treatment is warranted at that economic threshold. Note the variation in y-axis at different economic thresholds.

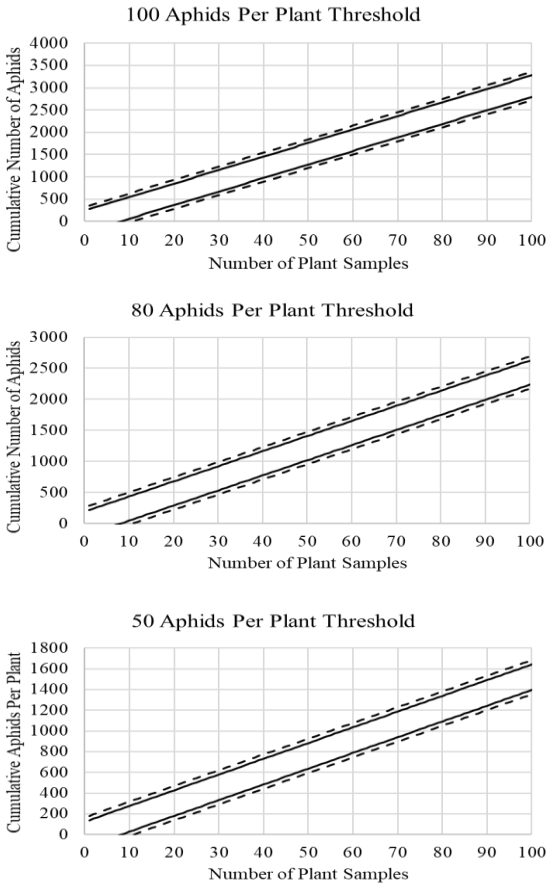


Figure 6: **Wald's (1947) SPRT stop-lines for classification of *M. sacchari* at treatable and non-treatable levels for the southern region.** The upper and lower limits of the stop-lines are dashed at 5% and solid at 10% alpha and beta error. If cumulative aphids at a given number of plant samples is above the upper limit insecticidal treatment is warranted at that threshold. If the minimum sample size of 20 plants had less than the lower limit of cumulative aphids, no treatment is warranted at that economic threshold. Note the variation in y-axis at different economic thresholds.

While often used for binomial sequential sampling (Giles et al. 2000, Pedigo and Buntin 1994, Prager et al. 2014, Severtson et al. 2016), Wald's (1947) sequential sampling probability ratio (SPRT) stop-lines for enumerative sequential sampling plans can be utilized as well (Carvalho et al. 2007, Peng and Brewer 1996). In fact, for insects that fit a known distribution pattern (random, aggregated, or regular) Wald's SPRT is preferred over Iwao's (1985) confidence interval method (Pedigo and Buntin 1994). Based on *M. sacchari's* TPL value and adjusted common k value, the population

distribution is aggregated. It is for this reason Wald's SPRT stop-lines for negative binomial distributions was deemed the best fit model to classify intensities above or below the selected ETs.

As was seen in the sequential sampling for population estimation, only in the southern region does this sequential sampling plan seem moderately applicable in a field scouting situation. The northern region still required more sampling at higher cumulative counts than may be plausible at the field level when accounting for time and accuracy of counts well over 1000. Twenty samples, the minimum number of samples required, is still pushing the higher end for efficient sampling protocols.

Conclusions

This study provided three different enumerative sampling protocols to monitor *M. sacchari* in grain sorghum. The first protocol was a fixed sample size plan that provided the minimum required number of samples needed to reach a mean, pre-selected intensity at 0.10 and 0.25 precision. Essentially, the number of samples required to estimate a population at a given mean was too high for practical application for either research or scouting purposes at a 0.10 precision level. At a 0.25 precision level, fixed sample sizes were reasonable for either region, for research application, when *M. sacchari* intensities were high. Only in the southern region at high intensities or using a high economic threshold would the fixed sample size plan at a 0.25 precision level align with current practices for scouting (Szczepaniec, 2018) and be practical for application at a field level.

The second enumerative sampling plan developed in this study was the sequential sampling to estimate population density within a field at a fixed precision level. While

the calculated stop-lines for fixed precision at the 0.25 level seemed practical at a research level, the validation of this plan demonstrated higher required sample sizes then originally predicted and high variation in precision depending on field intensity.

The third protocol was sequential sampling for classification of a population using economic thresholds. This sampling plan, similarly, to the other two sampling plans showed that in the southern region these stop-lines may be practical for in-field use. However, the sequential sampling for classification, like the other two sampling plans, did not appear practical for use in the northern region.

Overall, this study was a testament to how important defining distribution/aggregation patterns can be and how much they can affect the outcome of a sampling plan. Although both regions have definitively aggregated populations, as aphids often do, the difference in the northern vs the southern region resulted in only the southern region having a sampling plan that could be practical for field use. To that effect, even in the southern region, at threshold level population intensities the sampler would still have to take between 20-40 plant samples and keep track over well over 1000 cumulative aphid counts. If cumulative aphid counts are done with high accuracy this would take even an experienced sampler a very long time. The time needed to estimate or classify a population in this way would be costly in an integrated pest management program (Babu and Reisig 2018). This study, although providing insight to what it would take to precisely estimate a field population, provides evidence to why a binomial sequential sampling plan to classify a population may be ideal for the monitoring and management of *M. sacchari*.

CHAPTER V

DEVELOPMENT OF BINOMIAL SEQUENTIAL SAMPLING PLANS FOR SUGARCANE APHID IN COMMERCIAL GRAIN SORGHUM

Abstract

Sugarcane aphid, *Melanaphis sacchari* Zehntner, is a significant economic pest in grain sorghum in the Southeastern US and southern Great Plains. A collaborative study led by Oklahoma State University was conducted to develop an effective scouting plan for *M. sacchari* that allows growers and consultants to quickly determine when an economic threshold has been met. From 2016-2017, *M. sacchari* was sampled from commercial sorghum fields that included more than 331 sampling events over 140 locations in six states (OK, KS, TX, AR, LA, MS). Tally threshold regressions were analyzed to define the relationship between the mean *M. sacchari* density per leaf and proportion of plants infested. After the fitness and practicality of the model was considered, tally thresholds of 50 and 100 aphids per plant were selected. Wald's sequential probability ratio test (SPRT) was used to determine stop lines for both sampling plans, which ranged from 10-24 plant samples per sampling event, with an average of 11 plant samples per sampling event, depending on state, action threshold, and error level. The binomial sampling plans were validated using 48 externally sampled fields analyzed with resampling for validation of sampling plans (RVSP) software. An in-field sampling tool was developed using the tally threshold of 50 *M. sacchari.*

This study demonstrated that a control decision for *M. sacchari* with low error could be made with an average of 11 samples in sorghum fields across all states and action thresholds. Most importantly, this study provided a dynamic sampling plan for *M. sacchari* in sorghum that is operational for any location, yield goal, or treatment plan.

Introduction

In 2013, *Melanaphis sacchari* became a severe economic pest of grain sorghum and scientists from several disciplines responded to identify and develop management tools for this pest (Bowling et al. 2016, Armstrong et al. 2017, Brewer et al. 2019). Host plant resistance, adaptions in cultural practice, and chemical control have shown to reduce the destruction *M. sacchari* can cause. However, there has been no research-based, rapid scouting plan to determine when *M. sacchari* is above or below an economic threshold (ET).

Sampling plans are typically designed for detection of an organism, for estimating population density of an organism or for making decision about whether pest abundance is above or below a critical threshold (Moon and Wilson 2009). A sampling plan that combines early detection and objective monitoring of pest infestation levels is essential for administering effective control (Hodgson et al. 2005). A sequential sampling plan offers a flexible, statistically precise, and temporally efficient method for classification of insect density as it relates to a threshold density (Moon and Wilson 2009). Such plans are available for aphids in other crops (Naranjo and Hutchison 1997, Giles et al. 2000, Hodgson et al. 2005, Pedigo and Rice 2006, Severtson et al. 2016). Integrating a

binomial, or presence/absence, component to a sequential sampling plan increases the speed of sampling by removing the need to enumerate the individual aphids on a sample unit (Pedigo and Buntin 1994). Binomial sequential sampling plans are regarded as ideal for scouting with intent to make a treatment decision due to their fast and reliable nature (Pedigo and Rice 2006).

Early sampling recommendations for *M. sacchari* were rudimentary and control decisions were based on a percent of plants with substantial honeydew on them (Catchot et al. 2015). Two more recent *M. sacchari* sampling protocols are based on an intensity per-leaf economic threshold or by a growth stage "threshold". The per-leaf threshold sampling protocol directs the scout to estimate the aphid intensity on an upper most and lower-most leaf on ten randomly selected plants, in four different locations within a field (Biles 2018). If the average *M. sacchari* per leaf is more than 50, the recommendation is to chemically treat within three days (Biles 2018, Gordy et al. 2018). This sampling method is based on published research by Szczepaniec (2018) done exclusively in the high-plains of Texas. While the research objectives were largely to determine a yield-based economic threshold, no sampling protocol was discussed (Szczepaniec 2018). This leaves the question of why 10 samples from four stops of ten plants are needed.

Another newly developed "threshold" based sampling plan incorporated growth stage. The sampling plan recommends treatment if 20-30% of plants (based on a two-leaf sample of an upper-most and lower-most) are infested with 50 or more *M. sacchari* (Biles 2018, Gordy et al. 2018). This sampling plan, while binomial, is not sequential and

requires four stops of ten randomly selected plants making a total of 40 plant samples (Biles 2018, Gordy et al. 2018). Additionally, it is not adjustable to variable economic thresholds. Multiple other scouting protocols and recommended thresholds exist ranging from 40-60 samples, 20-30% infested with 25-125 sugarcane aphids per leaf or per plant (Brewer and Gordy 2016, Royer 2016, Armstrong et al. 2017, Elliott et al. 2017, Sorghum Checkoff 2017 Royer, 2018). Despite these recommendations, there remains no refereed study of a sampling protocol that encompasses multiple states, sites, and growth stages to produce a rapid classification protocol for *M. sacchari*.

The lack of a rapid decision tool in an integrated pest management program can result in yield loss caused by undetected aphid intensities that well exceed the economic injury level (EIL) or unnecessary chemical treatment when aphid intensities are well below an established economic threshold (ET) (Ahuja et al. 2015). The objective for this study was to develop a research-based, binomial sequential sampling plan for *M. sacchari,* that is adapted for multiple growth stages of sorghum over a broad geographical area. Such a tool can help producers determine when an ET had been met in order to make a treatment decision quickly with high precision.

Materials and Methods

Throughout the 2016 and 2017 growing seasons data were collected from 281 sampling event that took place at 134 locations across Kansas, Oklahoma, Texas, and Arkansas. All samples were collected from commercial sorghum fields using a stratified sampling protocol in a 3x3 cell grid scaling 90m X 90m. Within each 30m X 30m cell,

three adjacent plants within a row from two randomly selected stops were examined. Each plant sample consisted of a complete enumeration of *M. sacchari* from two leaves. Each sampling event consisted of 54 plant samples. A complete description of the standardized sampling procedure is outlined in chapter two.

In addition to the 281 sampling events described above, an additional 48 sampling events were used for validation of the sampling protocol. These added data sets were externally sampled in Kansas, Oklahoma, Texas, Louisiana, and Mississippi. Fields sampled in Louisiana and Mississippi where completed using the stratified sampling protocol described above. The fields sampled in Kansas, Oklahoma, and Texas were done using a modified version of the sampling plan with inverse "U" shaped pattern instead of a grid. In all sampling events, three consecutive plants within a row were enumerated per stop, and stops were within 30m of one another. Each sampling event began at an edge of the field and moved inward. All sorghum growth stages were represented in the data sets used for development and validation.

Development of a Tally Threshold and Binomial Sequential Sampling Plan

Using equation one (Pedigo and Buntin 1994, Giles et al. 2000) relationships between the proportion of infested plants with ≥T aphids (P_T) and the mean (m) number of aphids per plant based on a two leaf sample were evaluated for fitness using the coefficient of determination (r^2) (Table 9). The parameters for α and ƀ for ln(m) and ln ($1-P_T$) were derived from regression (PROC REG, SAS 9.4).

$$\boldsymbol{Ln(m) = \alpha + \beta ln\ (-ln\ [1-P_T])} \qquad \textbf{(1)}$$

To calculate stop lines for the binomial sequential sampling plan were based on Wald's sequential probability ratio test (SPRT) was used (equation 2) (Wald 1947, Alyousuf 2018). Tally thresholds of 50 and 100 were used due to a relatively high r-squared value from regression analysis and the visual ease of identification of 50 and 100 or more aphids (Morgan et al. 2014, Gordy et al. 2018, Szczepaniec 2018).

$$T_U(n) = Bn + A \qquad (2)$$

$$T_L(n) = Bn - C$$

$$B = \ln[(1-P_0)/(1-P_1)] / \ln[P_1(1-P_0)/(P_0(1-P_1))]$$

$$A = \ln[(1-\beta)/\alpha] / \ln[P_1(1-P_0)/P_0(1-P_1))]$$

$$C = \ln[\beta/(1-\alpha)] / \ln[P_1(1-P_0)/P_0(1-P_1))]$$

In equation (2) n = total number of plant samples; $T_{(n)}$ = the total number of plants infested with at least T aphids; P_1 (upper parameter) and P_0 (lower parameter) were set at a maximum of ±0.15 of action thresholds set at 0.2, 0.3, 0.4, and 0.5 for T = 50 and 0.1, 0.2, 0.3, and 0.4 for a T = 100. Where B = slope and A and C are the upper and lower intercepts. Type I and type II error were calculated equally at α and β or 5% and 10% probability, with a type I error being defined as treatment when the actual aphid intensity is below the economic threshold and type II error being defined as no treatment when the actual aphid intensity is above the economic threshold.

After stop lines were developed, the binomial sequential sampling plan was validated using Resampling for Validation of Sampling Plans (RVSP) software at T=50 and T= 100 (Naranjo and Hutchison 1997). Parameters for validation iterations were set the same as the development of the stop lines with P_1 (upper) and P_0 (lower) boundaries at ±0.15 for all action thresholds and T values. Alpha and beta error were set at 0.05 and 0.10 for each test. Software preformed 500 iterative samplings on all 50 of the externally sampled fields described above. Average sample number (ASN) and operating characteristics (OC) were determined for all action thresholds at each tally threshold. ASN and OC curves were generated in Microsoft Office Excel by plotting the RVSP output per sampling event against the proportion of infested plants with T number of aphids.

Results

Tally Threshold Determination

Melanaphis sacchari population intensity ranged from 0.01-1109.11 aphids per plant in the development set, and 0.32-2415.74 aphids per plant in the validation data set. In total, fifteen different regression models were initially evaluated ranging from a tally of 1 to 250 aphids per plant (Table 9). The tally thresholds (T) that best fit the regression model between proportion infested with T aphids and the mean number of aphids per plant were T = 160, 165, and 170 (r^2=0.90). The least fit model was T = 1 (r^2=0.78). After evaluating the initial tally threshold models, values were selected for re-evaluation based on having goodness of fit and by having a value that was practical for scouting. A ceiling mean number of aphids per plant was set at ≤ 215 aphids (Table 10). By limiting the

mean number of aphids per plant, we removed all fields that had aphid densities that were well beyond any realistic economic injury level (EIL). The remaining fields in the data set were ones that were at or approaching any reported economic threshold as to make decision making more realistic and useful for the sampler (Brewer and Gordy 2016, Szczepaniec 2018). After re-analyzing the data with the mean aphids per plant limitation the best fit model was a tally threshold value of 160 (r^2=0.85). The tally threshold of one aphid was, again, the least fit model (r^2=0.52).

Table 9: Predicted relationship between the proportion of plants infested with selected tally thresholds (T) of *M. sacchari* and the mean intensity of *M. sacchari* per plant

T[A]	N[B]	α±SE	b±SE	MSE[C]	r^2
1	250	2.58±0.10	1.43±0.05	1.06	0.78
5	217	3.69±0.09	1.27±0.04	0.83	0.83
10	187	4.24±0.10	1.18±0.04	0.74	0.85
25	139	4.92±0.11	1.05±0.04	0.73	0.84
50	106	5.35±0.11	1.00±0.04	0.63	0.86
75	94	5.65±0.12	0.97±0.04	0.58	0.86
100	84	5.84±0.12	0.94±0.04	0.50	0.88
150	70	6.02±0.10	0.87±0.04	0.41	0.89
155	68	6.04±0.11	0.84±0.04	0.44	0.87
160	67	6.12±0.11	0.89±0.04	0.42	0.90
165	65	6.11±0.10	0.87±0.04	0.40	0.90
170	65	6.13±0.10	0.87±0.04	0.40	0.90
175	65	6.13±0.11	0.86±0.04	0.40	0.89
200	61	6.20±0.11	0.86±0.04	0.40	0.89
250	58	6.38±0.13	0.85±0.05	0.44	0.86

Table shows results of regression analysis of log of P_T by log of the mean where P_T is the proportion of plants infested with T aphids, and the mean is the average intensity per plant by sampling event of 54 two plant samples. A= the number of aphids present to be considered infested; B= number of sampling events; C= mean standard error.

Table 10: Predicted relationships between the proportion of plants infested with selected tally thresholds (T) of *M. sacchari* and the mean (limited to ≥215) intensity of *M. sacchari* per plant

T	n	α±SE	b±SE	MSE	r^2
1	147	2.55±0.10	1.01±0.08	1.04	0.52
50	96	5.24±0.14	0.97±0.05	0.63	0.81
75	84	5.60±0.15	0.94±0.05	0.57	0.81
100	74	5.76±0.15	0.92±0.05	0.50	0.82
150	60	5.90±0.15	0.83±0.05	0.40	0.83
160	55	6.00±0.15	0.84±0.05	0.37	0.85
200	51	6.03±0.16	0.81±0.05	0.39	0.82

Table shows results of regression analysis of log of P_T by log of the mean where P_T is the proportion of plants infested with T aphids, and the mean is the average intensity per plant, limited to averages no greater than 215 SCA, by sampling event of 54 two plant samples. A= the number of aphids present to be considered infested; B= number of sampling events; C= mean standard error

To evaluate which tally thresholds should be developed further, simple scatter plots were made comparing proportion infested with T number of aphids to the field average number of aphids per plant based on the two-leaf sample (Figure 7). The addition of a logarithmic trendline provided better visualization of which tally thresholds would relate to potential thresholds by providing a point of reference for where percent of plants infested with T aphids meets an average aphid intensity per plant. For example, if an economic threshold was determined to be an average of 100 aphids per plant, the sampler could determine that threshold was met when 30% of plants inspected had 50 or more aphids, whereas with a high T value just over 10% had to be deemed infested to be considered at a treatable level. The models selected were based on a two-leaf sampling of 54 plants per sampling event. If a tally threshold of 160 aphids per plant was considered, a treatment decision could be made in as few as 6 plants. Because it was decided to

require a minimum sample size of 10 plants before a decision could be made, sampling plans for 150 and 160 aphids per plant were discontinued and efforts were continued to develop plans using tally thresholds of 50 and 100 aphids per plant.

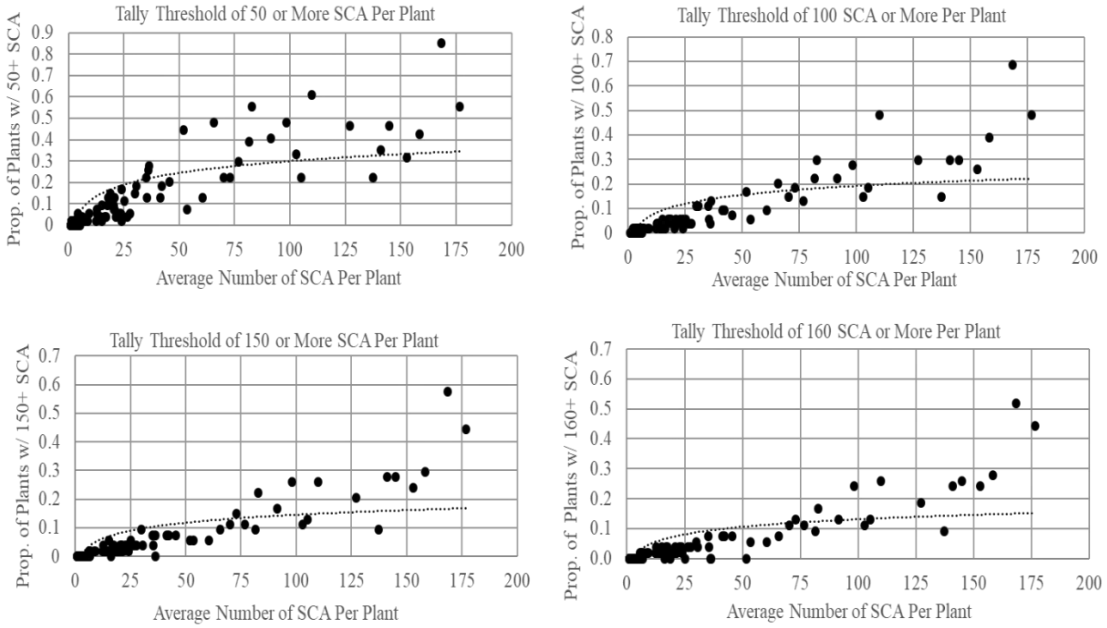


Figure 7: Empirical relationship curves with varying T-values. Empirical relationship curves between the proportion of plants infested with 50,100,150, and 160 or more *M. sacchari* and the average number of *M. sacchari* per plant.

Calculations of Wald's SPRT Stop-Lines

Once tally threshold values (50 and 100) were selected for further development, stop lines were calculated for them at varying action thresholds (Figures 8 and 9) using equation 2 from materials and methods. The action thresholds chosen for the tally threshold of 50 aphids per plant relate to mean aphid intensities per plant of 25 (20% infested), 75 (30% infested), 100 (40% infested), and 150 (50% or higher infestation). The action thresholds chosen for the tally threshold of 100 aphids per plant relate to the mean aphid intensities per plant of 25 (10% infested), 75 (20% infested), 100 (30% infested), and 150 (40% or higher infestation). These tally thresholds assimilated as many data points as possible over a range of aphid population intensities and accommodated the variable action thresholds listed in each state. This allowed for construction of a more dynamic and adjustable sampling protocol as economic thresholds change (Brewer and Gordy 2016).

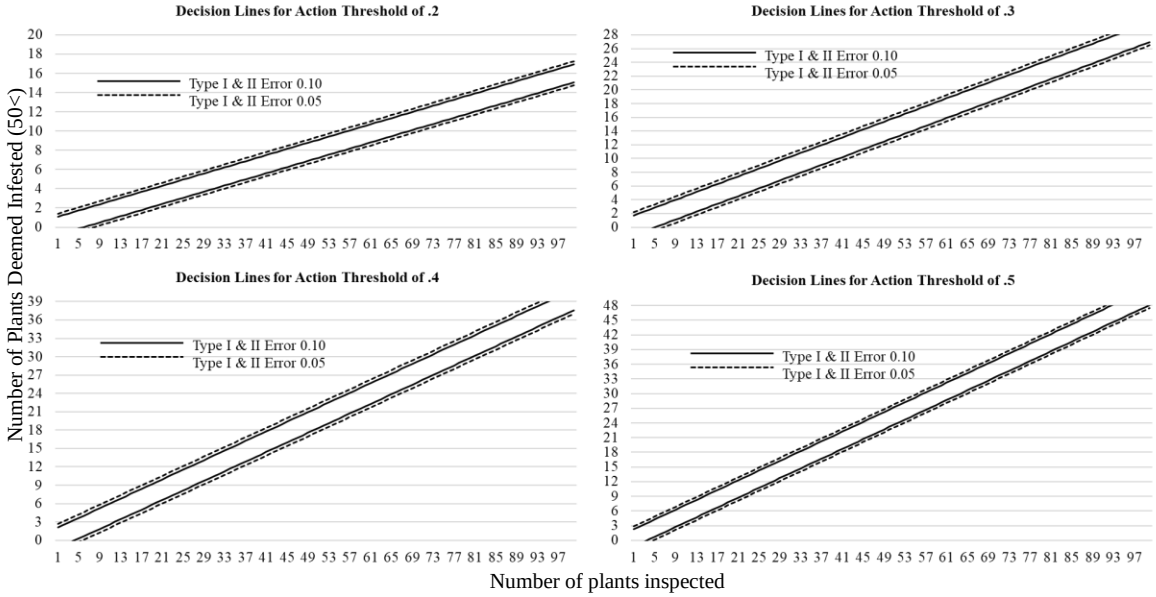


Figure 8: : **Decision stop-lines for tally threshold of 50 at varying ATs.**Decision stop lines at an action threshold of 0.2,0.3, 0.4, anf 0.5 for a tally threshold of 50 or more aphids per plant. Calculated using equation 2 Wald's SPRT (1949). Type one and type two error are reported at levels 0.10 and 0.05.

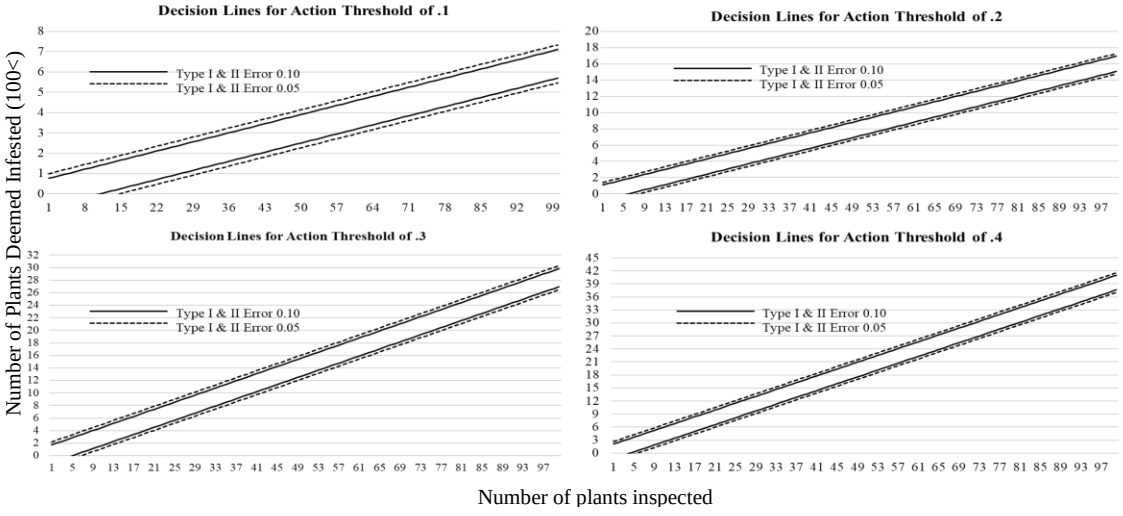


Figure 9: : **Decision stop-lines for tally threshold of 100 at varying ATs**. Decision stop lines at an action threshold of 0.2,0.3, 0.4, and 0.5 for a tally threshold of 100 or more aphids per plant. Calculated using equation 2 Wald's SPRT (1949). Type one and

Validation of Sampling Protocols

The results from the validation software (RVSP) were promising due to low variability in average sample number (ASN) despite changes in both error levels and action thresholds (Table 11). Despite dramatic differences in geographic location and action thresholds, the number of samples necessary to make a treatment decision fell well below the number required for other sampling protocols even at very low error rates. At a type I and type II error level of 0.10 for both tally thresholds the range for ASN over 500 sampling iterations was between 10 and 12 plant samples with 11 samples being the most frequent across all action thresholds. For a type I and II error level of 0.05 for both tally thresholds the ASN in 500 sampling iterations ranged from 10 to 15 samples with 11 remaining the most common ASN across all action thresholds. The highest probability of not treating was 91% determined by the average operation characteristic (OC) in tally threshold of 100 at action thresholds 0.3 and 0.4. The lowest probability of not treating based on the average OC was 70% at the lowest action threshold and T of 100 (Table 11). All operation characteristic averages and average sample number curves can be examined in figures 10 and 11.

Table 11: RSVP Wald's SPRT validation of Binomial Sequential Sampling Plans

Tally Threshold (total *M. sacchari*/plant)	Action Thresholds	Average Statistics for 500 Sampling Iterations			
		OC		ASN	
		0.10 (n=47)	0.05 (n=46)	0.10 (n=47)	0.05 (n=46)
100	0.1	0.71	0.70	12	15
100	0.2	0.84	0.86	11	11
100	0.3	0.91	0.90	11	11
100	0.4	0.91	0.91	10	11
50	0.2	0.74	0.76	11	12
50	0.3	0.86	0.86	11	12
50	0.4	0.87	0.87	11	11
50	0.5	0.89	0.89	10	10

Table portrays predicted values of the 500 sampling iterations created by the RVSP validation software on 50 external sampling events. Where OC stands for operating characteristic or the probability of not treating, and ASN stands for average sample number or the number of plants the sampling would have to evaluate on average

Discussion

This study developed two dynamic binomial sequential sampling protocols that use the presence or absence of 50 and 100 *M. sacchari* per plant. The protocols are consistent with sampling methods recommended by Brewer and Gordy (2016), Biles (2018), and Gordy et al. (2018) (e.g. enumeration of a two-leaf sample unit to predict the average number of aphids per plant). The biggest differences between the sampling protocols developed for this study and those recommended by Biles (2018) are that the former was developed over a large geographic area, requires fewer samples to make a treatment decision, is adaptable to a variety of action thresholds, and specifies how to sample across that large geographic area. Regarding the very low average number of

samples required, this may be explained by the variation in means per sampling event being so polarized. Very high aphids per leaf or very low aphids per leaf was observed and reflected in both the data used to build the sampling plan and to validate it. The low representation over all sampling events of intensities close to threshold, could explain why eleven samples is enough to make a treatment decision. Additionally, the NANOVA in Chapter III demonstrated that there is low variance in aphid counts between cells, since there are six samples per cell, by essentially sampling two cells, one may be sampling enough to confirm a treatment decision.

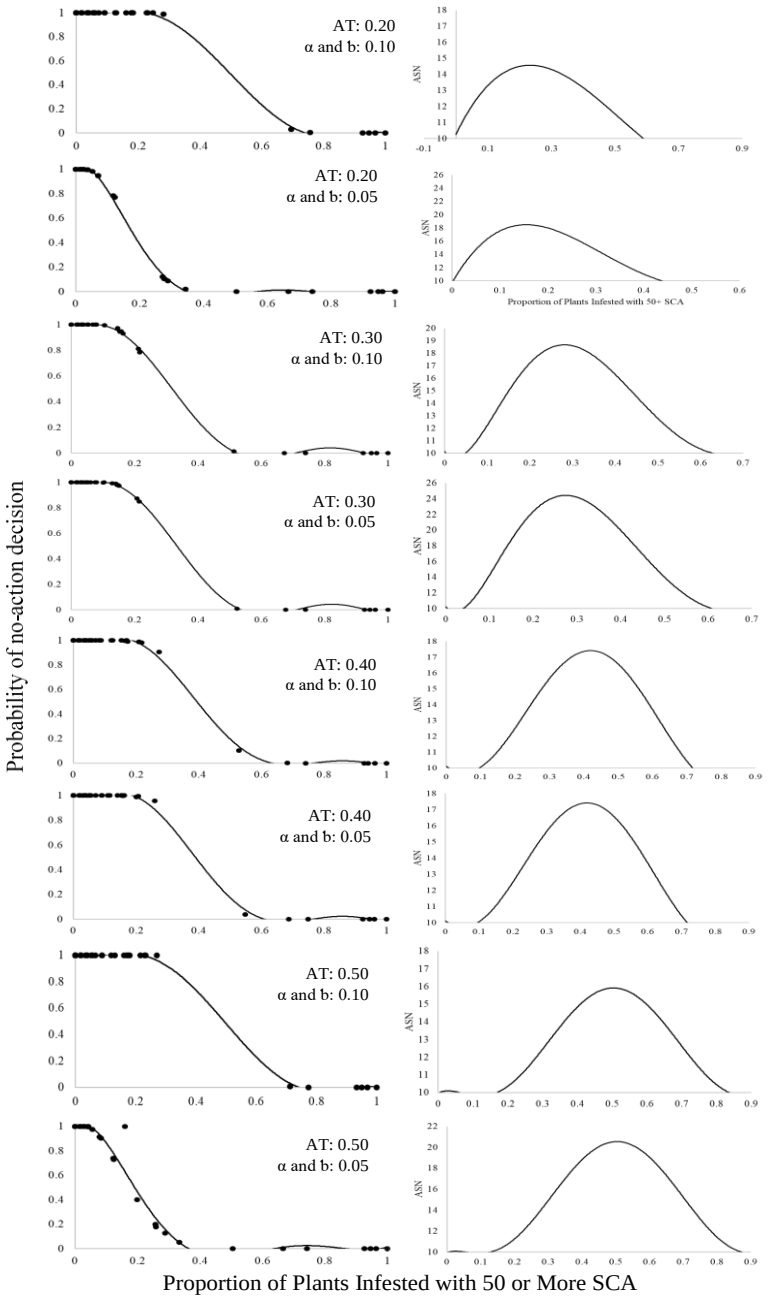


Figure 10: Operation characteristic and average sample number curves for T=50
Operation characteristic and average sample number (ASN) curves for tally threshold of 50 aphids or more. Varying action thresholds (AT) and alpha and beta error are included per graph pair. Both curves are based on 500 computer generated sampling iterations using 48 externally sampled fields.

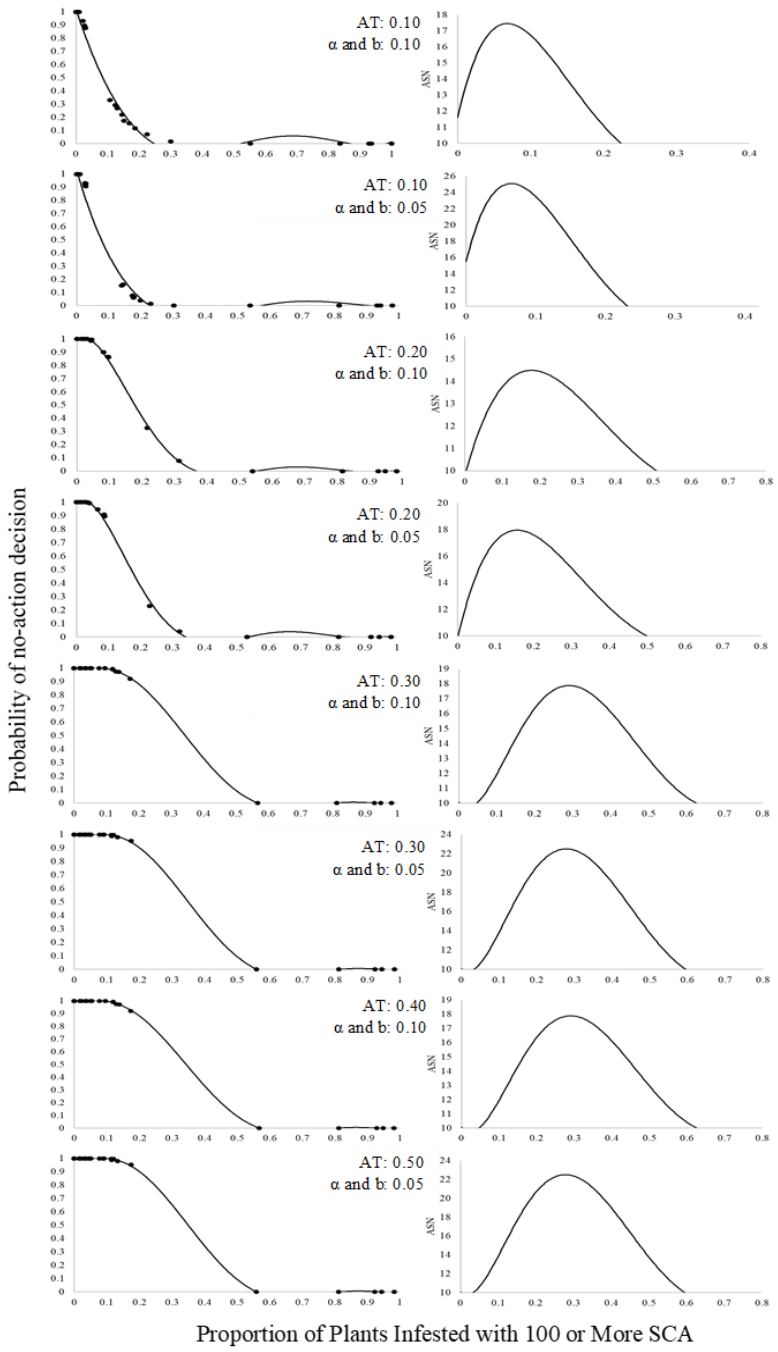


Figure 11: **Operation characteristic and average sample number curves for T=100**. Operation characteristic and average sample number (ASN) curves for tally threshold of 100 aphids or more. Varying action thresholds (AT) and alpha and beta error are included per graph pair. Both curves are based on 500 computer generated sampling iterations using 48 externally sampled fields.

These sampling protocols were developed from data spanning two growing seasons from six states, which is a highly robust data set compared with data used to develop binomial sequential sampling plans for row crop pests used today (Giles et al. 2000, Hodgson et al. 2005, Severtson et al. 2016). Also, the sampling protocol described in this study requires fewer samples to make a treatment decision compared with currently recommended sampling protocols for *M. sacchari* (Brewer and Gordy 2016, Biles 2018, Gordy et al. 2018). Based on the RVSP validation analysis, a treatment decision could be made with a minimum of 10 samples and a maximum of around 22 plant samples (Figures 10 and 11, Table 11). Even if the maximum number of samples was required in a field with an aphid intensity very close to threshold, the user of this protocol would sample only one half of the plants that the other protocols currently require. Because this sampling recommendation requires three consecutive plants per stop, the minimum sample number needed would be twelve plants (four stops of 3 plants) which only increases the precision of a treat/don't treat decision.

Secondly, this study not only provides two decision-based sampling protocols using two separate tally thresholds, but also includes four action thresholds per sampling protocol. The extensive range in action thresholds from 10% infested to 50% infested, or a plant average of 25-150 aphids, allows the user to adjust the sampling protocol to accommodate varying ET's, depending on the year, location, cost of treatment, commodity price, and personal yield goals. The protocol developed was built or validated using over 300 sampling events from more than 140 different sites across six states and

included data from all growth stages. The strong relationships seen within the models of percent infested with “T” aphid’s vs mean number of aphids per plant allows for confidence that either sampling protocol to determine precisely if an insecticide treatment is needed anytime during the growing season, across multiple states, and for multiple action thresholds.

Lastly, based on previous within field distribution data reported in Chapter IV, we can recommend best practices when scouting to increase precision. Using the data from this study combined with our previous study that describes within field and within plant distribution patterns, a sampling program is recommended to determine when an action threshold has been met. First, begin sampling at the edge of a field which should be no larger than 16.2-hectares, based on previously examined variance components. If field is larger than 16.2-hectares, additional samples will be necessary for every 16.2-hectares. One plant sample should include two 90% green leaves from the mid canopy, excluding the upper and lower-most two leaves. Simultaneously, determine if these two leaves have 50 or more aphids combined, and keep track of all plants that have more than 50 aphids. These models are built upon the relationship of aphid numbers from two leaves and the proportion of plants that had 50 or more aphids in order to predict a plant average intensity. For this reason, two leaves should be evaluated simultaneously to match the relationships analyzed. Repeat this process on the next two consecutive plants to make a stop that comprises three plants in a row. Each stop should be spaced 30m of the previous stop using a sampling pattern in the shape of an inverted “U”. A decision could be made

after a minimum of four stops are made. An example of the field guide has been included in the appendix (Ch. 5: Appendix Figure 13).

Moving forward, the binomial sequential sampling protocol that estimates presence or absence of a T = 50 or more aphids per plant appears to be the best choice. It is congruent with recommendations that already exist in the literature and extension publications (Brewer and Gordy 2016, Biles 2018, Gordy et al. 2018, Szczepaniec 2018), so there is a higher chance for quick adaption by end users. Other T values could be used, but a recent study that evaluated how humans can visually sense numbers found that adults were able to easily identify black dots as being roughly fifty with low error margins compared to clumps of 80 or more (Morgan et al. 2014). The study concluded that while variables like texture, blurriness, contrast, and aggregation played a part in people's ability to number sense, people could usually identify roughly 50 dots with less than 10% error (Morgan et al. 2014). In conclusion, the integration of this binomial sequential sampling protocol, using the presence or absence of 50 or more *M. sacchari*, into existing integrated pest management systems will result in faster, more reliable treatment decisions. Thus, lowering the amount of unnecessary insecticide application saving time and money for producers.

CHAPTER VI

CONCLUSIONS

The objectives of this study were to:

1. Elucidate the within-field aphid count variance per sample universe and define within-plant distribution patterns of sugarcane aphid in commercial grain sorghum.
2. Develop the three most common enumerative sampling plans, (fixed sample size, sequential sampling for population density prediction, and sequential sampling for population classification) for *M. sacchari* in grain sorghum.
3. Develop a research-based, binomial sequential sampling plan for *M. sacchari*, that is adapted for multiple growth stages of sorghum over a broad geographical area.

For the first objective, nearly 300 sampling events occurred in 134 grain sorghum fields sampled in Kansas, Oklahoma, Texas, Arkansas, Louisiana, and Mississippi. Taylor's Power Law defined two significantly different dispersion patterns identififed by their mean to variance relationship. The regions were designated by geographic location; the "southern" region included South Texas and Arkansas, while the "nothern" regions included Northern Texas, Oklahoma, and Kansas.

The nested analysis of variance (NANOVA) demonstrated that multiple samples of three plants in a row within 30 meters of one another be used because it accounted for 80-98% of the within-field count variance. Lastly the within-plant canopy distribution results from this study showed that regardless of the state or growth stage, the middle of the plant canopy tended to be most predictive of the whole plant population. Due to correlation, more models are needed to improve the confidence of the results. Yet, the studies results are powerful enough to suggest the two-leaf sample for any of the developed sampling protocols should come from the middle of the plant canopy.

This study provided three different enumerative sampling protocols to monitor *M. sacchari* in grain sorghum. The first protocol was a fixed sample size plan that provided the minimum required number of samples needed to reach a mean, pre-selected intensity at 0.10 and 0.25 precision. Essentially, the number of samples required to estimate a population at a given mean was too high for practical application for either research or scouting purposes at a 0.10 precision level. At a 0.25 precision level, fixed sample sizes were reasonable for either region, for research application, when *M. sacchari* intensities

were high. Only in the southern region at high intensities or using a high economic threshold would the fixed sample size plan at a 0.25 precision level align with current practices for scouting (Szczepaniec, 2018) and be practical for application at a field level.

The second protocol was an enumerative sampling plan developed was the sequential sampling to estimate population density within a field at a fixed precision level. While the calculated stop-lines for fixed precision at the 0.25 level seemed practical at a research level, the validation of this plan demonstrated higher required sample sizes then originally predicted and high variation in precision depending on field intensity.

The third sampling protocol was sequential sampling for classification of a population using economic thresholds. This sampling plan, similarly, to the other two sampling plans showed that in the southern region these stop-lines may be practical for in-field use. However, the sequential sampling for classification, like the other two sampling plans, did not appear practical for use in the northern region.

To fulfill the third objective, a sequential binomial sampling plan was developed to expedite monitoring for treatable *M. sacchari* intensities. First, predictive tally threshold models were evaluated for goodness-of-fit using linear regression. After carefully considering model fitness and practicallity, tally thresholds of 50 and 100 aphids per plant were selected to best predict the mean SCA per field. Wald's (1947) sequential probability ratio test (SPRT) was used to generate stoplines for sampling events. Both sampling plans were then validated using resampling for validation of

sampling plans (RVSP) software (Naranjo and Hutchison 1997) that provided the operating characteristic and average sample number (ASN) for 48 externally sampled fields. Averages were collected from the 500 sampling itterations, with four different action thresholds, and two error rates. The ASN ranged from 10-24 plant samples with an average of 11 plant samples. Moving forward the developed stoplines for a presence/absence of 50 or more aphids will be used to creat a smart-phone application that will help producers and other samplers quickly determine when *M. sacchari* populations are above or below threshold.

REFERENCES

Ahuja, D. B., U. R. Ahuja, S. K. Singh, and N. Singh. 2015. Comparison of Integrated Pest Management approaches and conventional (non-IPM) practices in late-winter-season cauliflower in Northern India. Crop Prot. 78: 232-238.

Alyousuf, A. 2018. Sampling plans for aphids on winter canola. PhD Dissertation, Oklahoma State University, Stillwater, OK.

Armstrong, S. J., W. L. Rooney, G. C. Peterson, R. T. Villenueva, M. J. Brewer, and D. Sekula-Ortiz. 2015. Sugarcane aphid (Hemiptera: Aphididae): Host range and sorghum resistance including cross-resistance from greenbug sources. J. Econ. Entomol. 108: 576-582.

Babu, A., and D. D. Reisig. 2018. Developing a sampling plan for brown stink bug (Hemiptera: Pentatomidae) in field corn. J. Econ. Entomol. 111: 1915-1926. https://doi.org/10.1093/jee/toy144 (accessed 21 March 2019).

Backoulou, G. F., N. C. Elliott, K. L. Giles, T. M. Alves, M. J. Brewer, and M. Starek. 2018. Using multispectral imagery to map spatially variable sugarcane aphid Infestations in sorghum. Southwest. Entomol. 43: 37-44.

Bayoumy, M. H., R. Perumal, and J. P. Michaud. 2016. Comparative life histories of greenbugs and sugarcane aphids (Hemiptera: Aphididae) coinfesting susceptible and resistant sorghums. J. Econ. Entomol. 109: 385-391.

Belov, V. 2015. Species *Melanaphis sacchari* - Sugarcane Aphid, identification, images, and information for insects, spiders and their kin for the United States & Canada. BugGuide.net https://bugguide.net/node/view/15740. Iowa State University, Ames.

Bermingham, J., T. L.Wilkinson, 2009. Embryo nutrition in parthenogenetic viviparous aphids. Physiological Entomology, 34(2), 103-109. doi:10.1111/j.1365-3032.2008.00669.x

Bertone, R. 2018. Oklahoma's top 10 agricultural products: A glimpse at the states's top ag commodities, based on 2017 cash receipts. Farm Flavor. Retrieved from https://www.farmflavor.com/oklahoma/oklahoma-ag-products/oklahomas-top-agriculture-products/

Biles, S. 2018. Sugarcane aphids above threshold in sorghum, Mid-coast IPM. Texas A&M AgriLife Extention.

Blackman, R. L., and V. F. Eastop [eds.]. 2017. Aphids on the world's crops : an identification and information guide (2nd ed.). Chichester, West Sussex, England; New York: Wiley.

Bliss, C. I., & Owen, A. R. G. 1958. Negative binomial distributions with a common *k*. Biometricka, 45, 37-58.

Bowling, R. D., M. J. Brewer, D. L. Kerns, J. Gordy, N. Seiter, N. E. Elliott, G. D. Buntin, M. O. Way, T. A. Royer, S. Biles, and E. Maxson. 2016. Sugarcane aphid (Hemiptera: Aphididae): A new pest on sorghum in North America. Journal of Integrated Pest Management 7: 12. https//:DOI: 10.1093/jipm/pmw011.

Braendle, C., G. K. Davis, J. A. Brisson, and D. L. Stern. 2006. Wing dimorphism in aphids. Heredity 97: 192-199.

Brewer, M. J., & Gordy, J. 2016. Sugarcane aphid economic threshold and sampling update. http://ccag.tamu.edu/files/2016/02/2015brewergordywebinarversionfinal.pdf

Brewer, M. J., F. B. Peairs, and N. C. Elliott. 2019. Invasive cereal aphids of North America: Ecology and pest management. Annu. Rev. Entomol. 64:73-93.

Brewer, M. J., Way, M.O., Armstrong, J.S., Biles, S.P., Sekula, D., Swart, J.S., Crumley, C.R., Knutson, A.E., Villanueva, R.T., Parker, R.D., Odvody, G.N., Ragsdale, D.W. 2013. Outbreak of sorghum/sugarcane aphid on sorghum: First detections, distribution, and notes on management, Wheat, peanut, and other field crops research. Texas Plant Protection Conference, College Station, TX.

Buntin, D. 2015. Nine tips to control sugarcane aphid in sorghum in 2015. Southeast Farm Press Retrieved from http://www.southeastfarmpress.com/grains/9-tips-control-sugarcane-aphids-sorghum-2015 **t**controls wing polymorphism in males of the pea aphid, *Acyrthosiphon pisum* (Harris). Heredity, 89, 346-352. doi:10.1038/sj.hdy.6800146

Carvalho, M., A. Mexia, and L. Torres. 2007. *S*equential sampling plans for classifying *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) status in a cigarette factory on the Cape Verde islands. African Entomol. 15: 37-44. https://doi.org/10.4001/1021-3589-15.1.37. (accessed 21 March 2019).

Catchot, A., J. Gore, and D. Cook. 2015. Management guidelines for sugarcane aphids in MS grain sorghum, Mississippi crop situation. Mississippi State University, Starkville, MS.

Chong, P. W., and L. J. Graham. 2013. The 'Russian doll' approach: developing nested case-studies to support international comparative research in education. International J. Res. & Methods in Education 36: 23-32.

Colares, F., J. P. Michaud, C. L. Bain, and J. B. Torres. 2015a. Indigenous aphid predators show high levels of preadaptation to a novel prey, *Melanaphis sacchari* (Hemiptera: Aphididae). J. Econ. Entomol. 108: 2546-2555.

Colares, F., J. P. Michaud, C. L. Bain, and J. B. Torres. 2015b. Recruitment of aphidophagous arthropods to sorghum plants infested with *Melanaphis sacchari* and *Schizaphis graminum* (Hemiptera: Aphididae). Biological Control 90: 16-24.

David, S. K., & Sandhu, G. S. (1976). New oviparous morph on *Melanaphis sacchari* (Zehntner) on sorghum. Entomol., Rec. 88(28-29).

Delmotte, F., N. Leterme, J. P. Gauthier, C. Rispe, and J. C. Simon. 2002. Genetic architecture of sexual and asexual populations of the aphid *Rhopalosiphum padi* based on allozyme and microsatellite markers. Mol. Ecol. 11: 711-723.

Denmark, H. A. 1988. Sugarcane aphids in Florida. Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Entomology Curcular 302.

Dixon, A. F., and R. Kundu. 1998. Resource tracking in aphids: programmed reproductive strategies anticipate seasonal trends in habitat quality. Oecologia 114: 73-78.

Eastop, V.F. 1955. The periodicity of aphid flight in East Africa. East African Agriculture & Forestry Research Organisation Report.;pp 305–310.

Elliott, J. M. 1977. Some methods for the statistical analysis of samples of Benthic invertebrates vol. 25. Freshwater Biological Association.

Elliott, N. C., G. F. Backoulou, M. J. Brewer, and K. L. Giles. 2015. NDVI to detect sugarcane aphid injury to grain sorghum. J. Econ. Entomol. 108: 1452-1455.

Elliott, N.C., M. Brewer, N. Seiter, T. Royer, R. Bowling, G. Backoulou, J. Gordy, K. Giles, J. Lindenmayer, B. McCornack, and D. Kerns. 2017. Sugarcane aphid spatial distribution in grain sorghum fields. Southwest Entomol. 42: 27-35.

Elliott, N. C., K.L. Giles, T.A. Royer, S. D. Kindler, F. L. Tao, D. B. Jones, and G. W. Cuperus. 2003. Fixed precision sequential sampling plans for the greenbug and bird cherry-oat aphid (Homoptera : Aphididae) in winter wheat. J. Econ. Entomol. 96*:* 1585-1593. https://doi.org/10.1093/jee/96.5.1585 (accessed 21 March 2019)

Food and Agriculture Organization. 2008. The world sorghum and millet economies: facts, trends, and outlook. *In* E. A. S. Development; Food and Agriculture Organization

Giles, K. L., D. B. Jones, T. A. Royer, N. C. Elliott, and S. D. Kindler. 2003. Development of a sampling plan in winter wheat that estimates cereal aphid parasitism levels and predicts population suppression. J. Econ. Entomol. 96: 975-982.

Giles, K. L., McCornack, B. P., Royer, T. A., & Elliott, N. C. 2017. Incorporating biological control into IPM decision making. Curr. Opin. Insect Sci. 20: 84-89. doi:10.1016/j.cois.2017.03.009

Giles, K. L., T. A. Royer, N. C. Elliott, and S. D. Kindler. 2000. Development and validation of a binomial sequential sampling plan for the greenbug (Homoptera : Aphididae) infesting winter wheat in the southern plains. J. Econ. Entomol. 93: 1522-1530.

Gordy, J. W., M. J. Brewer, R. D. Bowling, G. D. Buntin, N. J. Seiter, D. L. Kerns, F. P. F. Reay-Jones, and M. O. Way. 2019. Development of economic thresholds for sugarcane aphid (Hemiptera: Aphididae) in susceptible grain sorghum hybrids. J. Econ. Entomol. https://doi.org/10.1093/jee/toz028

Gordy, J., M. Brewer, and M. O. Way. 2018. Comparison of tally-based thresholds and density-based thresholds for sugarcane aphid management, 2018 ESA, ESC, and ESBC Joint Annual Meeting Vancouver, Canada.

Green, R. H. 1970. On fixed precision level sequential sampling. Researches on Population Ecology, 12: 249-251.

Guo, S. S., M. Zhang, and T. X. Liu. 2016. Insulin-related peptide 5 is involved in regulating embryo development and biochemical composition in pea aphid with wing polyphenism. Front. Physiol. 7. https://doi.org/10.3389/fphys.2016.00031

Hardie, J., N. Gao, T. Timar, P. Sebok, and K. Honda. 1996. Precocene derivatives and aphid morphogenesis. Arch. Insect Biochem. 32: 493-501.

Harvey, T.L., and Hackerott, H.L. 1969. Recognition of a greenbug biotype injurious to sorghum. J. Econ. Entomol. 62: 776–779.

Hawkins, S., Williams, E., & Hodges, M. 2017. Grain sorghum production calendar. OSU extention fact sheets. Retrieved from http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Rendition-3261/unknown

Hewlett, J. A., A. Szczepaniec, and M. D. Eubanks. 2019. The effects of sugarcane aphid density in sorghum on predation by lady beetles and lacewings. Biological Control 129: 171-177.

Hodgson, E. W., E.C. Burkness, W.D. Hutchison, and D.W. Ragsdale. 2005. Enumerative and binomial sequential sampling plans for soybean aphid (Homoptera: Aphididae) in soybean, J. Econ. Entomol. 97: 2127-2136.

Ishikawa, A., H. Gotoh, T. Abe, and T. Miura. 2013. Juvenile hormone titer and wing-morph differentiation in the vetch aphid Megoura crassicauda. J. Insect Physiol. 59: 444-449.

Ishikawa, A., K. Ogawa, H. Gotoh, T. K. Walsh, D. Tagu, J. A. Brisson, C. Rispe, S. Jaubert-Possamai, T. Kanbe, T. Tsubota, T. Shiotsuki, and T. Miura. 2012. Juvenile hormone titre and related gene expression during the change of reproductive modes in the pea aphid. Insect Mol. Biol. 21: 49-60.

Kring, T. J., and F. E. Gilstrap. 1986. Beneficial role of corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae), in maintaining *Hippodamia* spp. (Coleoptera: Coccinellidae) in grain sorghum. Crop Protection 5: 125-128.

Kuno, E. 1969. A new method of sequential sampling to obtain the population estimates with a fixed level of precision Researches on Population Ecology 11: 127.

Lagos-Kutz, D., D. Voegtlin, J. Davis, and G. Hartman. 2018. Dispersal records of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), through the midwest suction trap network. Fla. Entomol. 101: 508-510.

Lampert, E.P. 1989. Seasonal abundance and within-plant distribution of aphids (Homoptera: Aphididae) in flue-cured tobacco. J. Econ. Entomol. 82: 114-118.

Lepš, J. 1993. Taylor's power law and the measurement of variation in the size of populations in space and time. Oikos 68: 349-356.

Leather, S. R. 2009. Analyses in insect ecology and management. Bulletin of Entomological Research, 87(2), 219-220. doi:10.1017/S0007485300027450

Lofton, J. 2019. Sorghum production and Oklahoma cropping systems**.** Personal communitation. Oklahoma State University, Stillwater, OK.

Lloyd, M. 1967. Mean crowding. Journal of Animal Ecology. 36: 1-30.

Luper, C., J. Criswell, T. Royer, and R. Kochenower. 2009. Crop profile for Oklahoma sorghum. https://ipmdata.ipmcenters.org/source_report.cfm?sectionid=40&sourceid=653

Matocha, M. A., J. Blumenthal, P. A. Baumann, and T. Isakeit. 2008. Crop profile for sorghum in TX. Texas AgriLife Extention College Station, TX.

Michaud, J. P. 2013. Sorghum midge: Implications for grain sorghum in central and western Kansas. Agronomy eUpdate(718).

Michaud, J. P., Bain, C., & Abdel-Wahab, A. (2018). Mortality of Sugarcane Aphid, Melanaphis sacchari (Zehntner, Hemiptera: Aphididae), at Low Temperatures. Journal of Economic Entomology, 111(5), 2496-2498. doi:10.1093/jee/toy195

Michels, G. J., and J. H. Matis. 2010. Corn leaf aphid, *Rhopalosiphum maidis*, as a key to greenbug, *Schizaphis graminum*, biological control in grain sorghum, *Sorghum bicolor*. . Texas A&M AgriLife Extention Amarillo, TX.

Milgroom, M. G. 2017. Population Biology of Plant Pathogens: Genetics, Ecology, and Evolution: APS Publications.

Moon, R. D., and L. T. Wilson. 2009. Sampling for detection, estimation, and IPM decision making. *In* E. B. Radcliffe, W. D. Hutchinson, and R. E. Cancelado (eds.), Integrated pest management: concepts, tactics, strategies and case studies: Cambridge University Press, NY.

Morgan, M. J., S. Raphael, M. S. Tibber, and S. C. Dakin. 2014. A texture-processing model of the visual sense of number. Proc. Royal Society B: Biological Sciences 281: 20141137. http://dx.doi.org/10.1098/rspb.2014.1137

Naranjo, S. E., and S. J. Castle 2010. Sequential sampling plans for estimating density of glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae) on citrus. Crop Protection, 29: 1363-1370. https://doi.org/10.1016/j.cropro.2010.07.003 (accessed 21 March 2019).

Naranjo, S. E., and W. D. Hutchison. 1997. Validation of arthropod sampling plans using a resampling approach: software and analysis. American Entomologist: 43: 48-57. https://doi.org/10.1093/ae/43.1.48. (accessed 21 March 2019).

Nibouche, S., B. Fartek, S. Mississipi, H. Delatte, B. Reynaud, and L. Costet. 2014. Low genetic diversity in *Melanaphis sacchari* aphid populations at the worldwide scale. PLoS One 9: e0106067

Nibouche, S., S. Mississipi, B. Fartek, H. Delatte, B. Reynaud, and L. Costet. 2015. Host plant specialization in the sugarcane aphid *Melanaphis sacchari.* PLoS One 10: e0143704.

Nibouche, S.S. L. Costlet, J.R. Holt, A. Jacobson, A. Pekarcik, J. Sadeyen, J.S. Armstrong, G.C. Peterson, N. McLaren, R.F. Medina. 2018. Invasion of sorghum in the Americas by a new sugarcane aphid (*Melanaphis sacchari*) superclone. PLoS ONE 13: e0196124.

Norris, R. F., Casewell-Chen, E. P., & Kogan, M. 2003. Pests, people, and integrated pest management In S. Helba (Ed.), Concepts of integrated pest management (pp. 1-14). NJ: Pearson Education Inc.

National Sorghum Producers. 2016. Sorghum 101. Retrieved from https://sorghumgrowers.com/sorghum-101/

Ogawa, K., and T. Miura. 2014a. Aphid polyphenisms: trans-generational developmental regulation through viviparity. Front. Physiol. 5:1 https://doi.org/10.3389/fphys.2014.00001

Parry, H. R. 2013. Cereal aphid movement: general principles and simulation modelling. Movement Ecology 1: 14. http://www.movementecologyjournal.com/content/1/1/14 (Accessed 26 Mar. 2019)

Peccoud, J., J. C. Simon, C. von Dohlen, A. Coeur d'acier, M. Plantegenest, F. Vanlerberghe-Masutti, and E. Jousselin. 2010. Evolution/Évolution. Comptes rendus - Biologies 333: 474-487.

Pedigo, L. P., & Buntin, G. D. 1994. Handbook of sampling methods for arthropods in agriculture. Boca Raton, Florida CRC Press LLC.

Pedigo, L. P., & Rice, M. E. 2006. Entomology and pest management (D. Yarnell Ed. Fifth ed.). Upper Saddle River, New Jersey: Pearson Education Inc.

Peng, C., and G.J. Brewer. 1996. Sequential sampling plans for the red sunflower seed weevil (Coleoptera: Curculionidae) in oilseed sunflower. J. Agric. Entomol. 13: 139-147. http://scentsoc.org/Volumes/JAE/v13/2/00132139.pdf (accessed 21 March 2019).

Prager, S. M., C.D. Butler, and J.T. Trumble. 2014. A binomial sequential sampling plan for *Bactericera cockerelli* (Hemiptera: Triozidae) in *Solanum lycopersicum* (Solanales: Solanacea) J. Econ. Entomol. 107: 838-845. https://doi.org/10.1603/EC13328 (accessed 21 March 2019).

Remaudiere, G., and S.E. Halbert. 1996. American species of the genus *Brachyunguis das* (Homoptera: Aphididae), including two new species. Proceedings of the Entomological Society of Washington, 98(2): 249-255.

Rimmer, J., C. Herman, B. Wingo, K. Fontaine, and T. Mehta. 2018. Methodological and clinical implications of a three-in-one Russian doll design for tracking health trajectories and improving health and function through innovative exercise treatments in adults with disability, vol. 18.

Royer, T. A. 2014. Damaging pests viewed in Oklahoma sorghum fields *In* D. Stotts [ed.]. Oklahoma State University agricultural extention, Stillwater, OK.

Royer, T. A. 2015. Oklahoma sorghum: Pest management-how to make a decision for this "horse race"?, AgFax.

Royer, T. A. 2016. Sugarcane aphid: *Melanaphis sacchari* (Zehntner), pp. 57-58 *in*: Royer, T.A. D Arnold and B. Hellie [eds]: Common insects of field crops in Oklahoma. E-1043. Oklahoma Cooperative Extension Service, Oklahoma State University, Stillwater.

Royer, T.A. 2018. Management of insect and mite pests in sorghum, CR-7170. Oklahoma Cooperative Extention Service, Oklahoma State University, Stillwater.

Severtson, D., K. Flower, and C. Nansen. 2016. Spatially-optimized sequential sampling plan for cabbage aphids *Brevicoryne brassicae* L. (Hemiptera: Aphididae) in canola fields. J. Econ. Entomol. 109: 1929-1935. http://doi.org/10.1093/jee/tow147 (accessed 21 March 2019)

Singh, B. U., P. G. Padmaja, and N. Seetharama. 2004. Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: a review. Crop Protection 23: 739-755.

Soreng, R. J., P. M. Peterson, K. Romaschenko, G. Davidse, F. O. Zuloaga, E. J. Judziewicz, T. S. Filgueiras, J. I. Davis, and O. Morrone. 2015. A worldwide phylogenetic classification of the Poaceae (Gramineae). J. System. and Evol. 53: 117-137.

Sorghum Checkoff. 2017. All about sorghum. https://www.sorghumcheckoff.com/all-about-sorghum. United Sorghum Checkoff Program, Lubbock, TX.

Stern, V. M., R. F. Smith, R. van den Bosch, and K. S. Hagen. 1959. The integrated control concept. J. Agr. Sci. 29: 81-101.

Summers, T. E. 1978. Sugarcane aphid, *Melanaphis sacchari*, pp. 496. Plant Pest Rept. Florida Coop.

Szczepaniec, A. 2018. Assessment of a density-based action threshold for suppression of sugarcane aphids, (Hemiptera: Aphididae), in the southern high plains. J. Econ. Entomol. 111: 2201-2207. http//doi.org/10.1093/jee/toy176. (accessed 21 March 2019)

Taylor, L. R. 1961. Aggregation, variance and the mean. Nature. 189: 732-735.

Tran, A. K., and R.L. Koch. 2017. Spatial patterns and sequential sampling plans for predators of A*phis glycines* (Hemiptera: Aphididae) in Minnesota soybean. Environ. Entomol.46: 663-673. http://doi.org/10.1093/ee/nvx040. (accessed 21 March 2019)

United States Department of Agriculture. 2018. World agriculture supply and demand estimates. Washington DC Retrieved from https://www.usda.gov/oce/commodity/wasde/index.htm.

USDA National Agricultural Statistics Service. 2017. Agricultural Statistics 2017. 512 pp.

USDA National Agricultural Statistics Service. 2018. Oklahoma Agricultural Statistics Bulletin. 83 pp.

Wald, A. 1947. Sequential analysis: J. Wiley & Sons, Incorporated.

Wallin, J. R., and Loonan, D. V. (1971). Low level jet winds, aphid vectors, local weather, and barley yellow dwarf virus outbreaks. Phytopathology 61: 1068-1070. https://www.apsnet.org/publications/phytopathology/backissues/Documents/1971Articles/Phyto61n09_1068.pdf (accessed 21 March 2019).

White, W.H, T.E. Reagan and D.G. Hall. 2001. *Melanaphis sacchari* (Homoptera: Aphididae), a sugarcane pest new to Louisiana. Fla. Entomol. 84: 435-436.

Wilbrink, G. 1922. An investigation on spread of the mosaic disease of sugarcane by aphids. Medid Procfst, 10 (Java Suikerind): 413-456.

Wilson, L. T., and P. M. Room. 1983. Clumping patterns of fruit and arthopods in cotton with implications for binomial sampling. Environ. Entomol. 12: 50-54. https://doi.org/10.1093/ee/12.1.50. (accessed 21 March 2019)

Wittgreve, R. 2017. Sweet sorghum FAQs. Retrieved from http://nssppa.org/Sweet_Sorghum_FAQs.html

Zera, A. J. 2016. Juvenile Hormone and the endocrine regulation of wing polymorphism in insects: new insights from circadian and functional-genomic studies in *Gryllus* crickets. Physiol. Entomol. 41: 313-326.

APPENDICES

CHAPTER III

AP Figure 12: Stratified sampling grid used to collect counts of *M. sacchari* in sorghum.

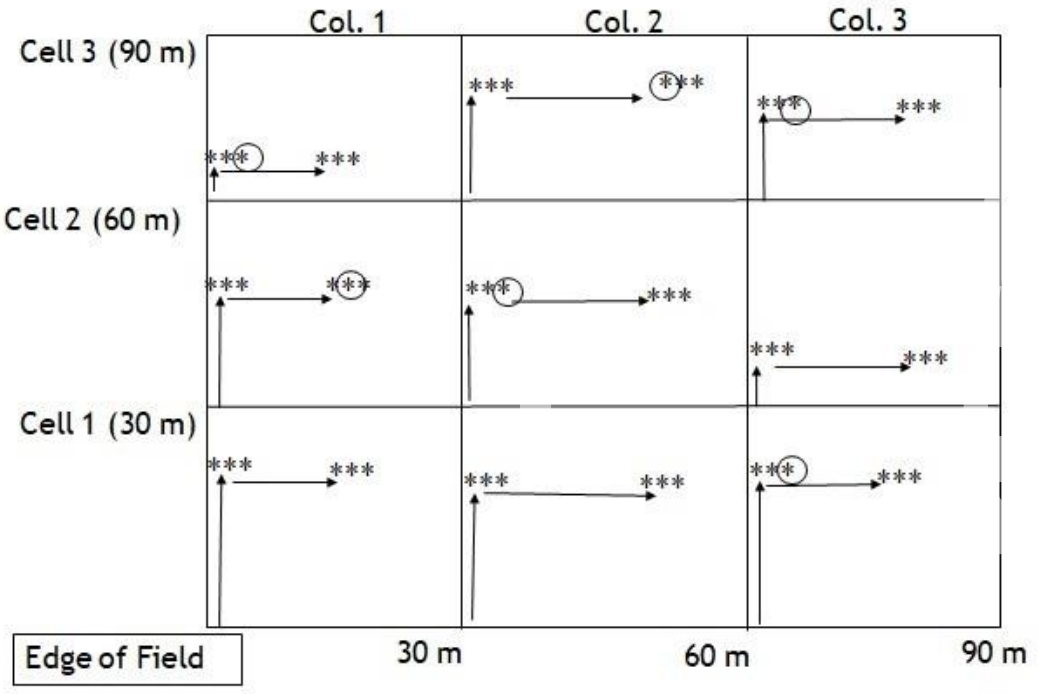


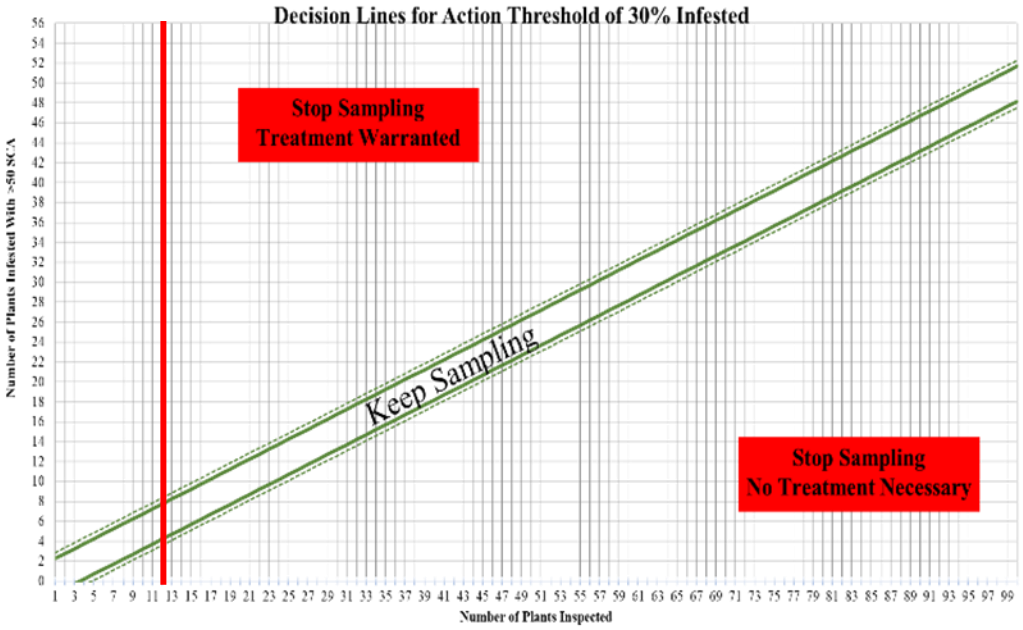
Figure illustrates example sampling event. Arrows indicate walking paths to first and second stops per cell; stars indicate plants evaluated; circled stars indicate whole plant enumeration. Distance to stops and whole plant enumerations were randomly selected and changed each sampling event.

AP Table 12: Within canopy distribution analysis using multiple regression.

State	Growth Stage	Canopy Position	Number of Plants	MSE	F-Statistic/P-Value	R^2
Kansas Oklahoma	**5**	**Middle**	**25**	**1.052**	**27.37/<0.0001**	**0.5434**
	1	**Middle**	**11**	**0.657**	**7.97/0.0199**	**0.4696**
	2	**Middle**	**21**	**1.313**	**58.67/<0.0001**	**0.7554**
	2	Lower	21	2.010	14.22/0.0013	0.4281
	3	**Upper[b]**	**77**	**1.701**	**845.90/<0.0001**	**0.9186**
	3	Middle[a]	77	4.258	72.01/<0.0001	0.4898
	3	Lower[b]	77	3.788	110.71/<0.0001	0.5962
	4	**Upper**	**36**	**1.420**	**79.21/<0.0001**	**0.6997**
	4	Middle	36	1.981	24.11/<0.0001	0.4149
	5	Upper[a]	98	8.990	312.12/<0.0001	0.7648
	5	**Middle[b]**	**98**	**5.725**	**910.30/<0.0001**	**0.9046**
	5	Lower[c]	98	13.810	76.96/<0.0001	0.4450
North Texas	2	Upper	69	1.954	64.78/<0.0001	0.4916
	2	Middle	69	1.746	97.98/<0.0001	0.5939
	2	**Lower**	**69**	**1.440**	**175.69/<0.0001**	**0.7239**
	3	**Upper**	**48**	**0.7067**	**51.82/<0.0001**	**0.5297**
	3	Middle	48	0.962	6.78/0.0124	0.1284
	3	Lower	48	0.984	4.49/0.0396	0.0889
	4	Upper	61	5.376	98.63/<0.0001	0.6257
	4	Middle	61	4.620	154.47/<0.0001	0.7236
	4	**Lower**	**61**	**3.071**	**424.09/<0.0001**	**0.8779**
	5	Upper[b]	42	5.754	74.73/<0.0001	0.6513
	5	Middle[b]	42	3.378	292.82/<0.0001	0.8798
	5	**Lower[a]**	**42**	**2.353**	**646.03/<0.0001**	**0.9417**
South Texas	1	Upper[b]	54	9.211	328.22/<0.0001	0.8632
	1	**Middle[a]**	**54**	**4.733**	**1387.93/<0.0001**	**0.9639**
	1	Lower[b]	54	12.373	158.72/<0.0001	0.7532
	2	**Upper**	**14**	**4.191**	**68.62/<0.0001**	**0.8511**
	2	Middle	14	4.572	55.74/<0.0001	0.8229
	2	Lower	14	6.487	21.66/0.0006	0.6434
	3	Upper	19	6.457	26.91/<0.0001	0.6129
	3	**Middle**	**19**	**3.002**	**186.15/<0.0001**	**0.9163**
	3	Lower	19	5.727	38.82/<0.0001	0.6954
	4	Upper	18	3.817	55.46/<0.0001	0.7761
	4	**Middle**	**18**	**3.276**	**81.00/<0.0001**	**0.8351**
	5	**Upper**	**20**	**7.953**	**51.56/<0.0001**	**0.7416**
	5	Middle	20	9.958	26.43/<0.0001	0.5949
	5	Lower	20	11.916	13.03/0.0020	0.4199

State	Growth Stage	Canopy Position	Number of Plants	MSE	F-Statistic/P-Value	R^2
Arkansas	1	Upper[d]	11	18.284	6.16/0.0349	0.4063
	1	**Middle[a]**	**11**	**4.693**	**221.13/<0.0001**	**0.9609**
	1	Lower[a]	11	5.030	191.28/<0.0001	0.9551
	3	Upper[b]	29	16.665	45.42/<0.0001	0.6272
	3	**Middle[a]**	**29**	**5.690**	**594.33/<0.0001**	**0.9565**
	3	Lower[c]	29	14.618	67.13/<0.0001	0.7132
	4	Upper[b]	10	11.000	6.79/0.0313	0.4592
	4	**Middle[a]**	**10**	**7.198**	**26.53/0.0009**	**0.7683**
	4	Lower[a]	10	9.110	13.56/0.0062	0.6290
	5	Upper	43	5.269	101.62/<0.0001	0.7125
	5	Middle	43	3.386	304.35/<0.0001	0.8813
	5	**Lower**	**43**	**2.964**	**409.53/<0.0001**	**0.9090**

Results of the multiple-regression analysis using state, growth stage, and position within the plant canopy, as predictors for the total aphids per plant, only models at or above a p-value of 0.05 were included in the table. In addition, the fixed-effects model results were included to show what states and growth stages had canopy positions that were significantly different (<0.05) from one another. Canopy positions that are denoted with a lower-case alphabetical superscript with different letters are significantly different from one another. All other models without a letter by the canopy position were not significantly different from one another.



Plants Sampled per 3-plant Stop (* =<50, * =>50)		Number of plants w/<50 SCA DON'T TREAT	Decision not Possible KEEP SAMPLING	Number of Plants w/50+ SCA TREAT
1-3	* * *	KEEP SAMPLING	KEEP SAMPLING	KEEP SAMPLING
4-6	* * *	KEEP SAMPLING	KEEP SAMPLING	KEEP SAMPLING
7-9	* * *	KEEP SAMPLING	KEEP SAMPLING	KEEP SAMPLING
10-12	* * *	2 or less, DON'T TREAT	3-7 KEEP SAMPLING	8+ TREAT
13-15	* * *	4 or less, DON'T TREAT	5-9 KEEP SAMPLING	10+ TREAT
16-18	* * *	6 or less, DON'T TREAT	7-11 KEEP SAMPLING	12+ TREAT
19-21	* * *	8 or less, DON'T TREAT	9-13 KEEP SAMPLING	14+ TREAT
22-24	* * *	9 or less, DON'T TREAT	10-14 KEEP SAMPLING	15+ TREAT
25-27	* * *	11 or less, DON'T TREAT	12-15 KEEP SAMPLING	16+ TREAT
28-30	* * *	12 or less, DON'T TREAT	13-17 KEEP SAMPLING	18+ TREAT
31-33	* * *	14 or less, DON'T TREAT	15-18 KEEP SAMPLING	19+ TREAT
34-36	* * *	15 or less, DON'T TREAT	16-19 KEEP SAMPLING	21+ TREAT
37-39	* * *	17or less, DON'T TREAT	18-21 KEEP SAMPLING	22+ TREAT
40-42	* * *	18 or less, DON'T TREAT	19-23 KEEP SAMPLING	24+ TREAT
43-45	* * *	19 or less, DON'T TREAT	20-25 KEEP SAMPLING	25+ TREAT
46-48	* * *	21 or less, DON'T TREAT	22-25 KEEP SAMPLING	26+ TREAT
49-51	* * *	22 or less, DON'T TREAT	23-27 KEEP SAMPLING	28+ TREAT
52-54	* * *	24 or less, DON'T TREAT	25-31 KEEP SAMPLING	32+ TREAT

AP Figure 13: **Sample field scouting tool.** Front and back of pocket field scouting tool using binomial sequential sampling protocol with presence or absence of 50 sugarcane aphids per two leaves combined at the 50% infested action threshold. Red line on the top figure represents

VITA

Jessica Carrie Lindenmayer

Candidate for the Degree of

Doctor of Philosophy

Dissertation: DEVELOPMENT OF A RESEARCH-BASED, USER FRIENDLY, RAPID SCOUTING PROCEDURE FOR THE INVASIVE SUGARCANE APHID *MELANAPHIS SACCHARI*, IN GRAIN SORGHUM

Major Field: Entomology

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Entomology at Oklahoma State University, Stillwater, Oklahoma in May, 2019.

Completed the requirements for the Master of Science in Entomology and Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in 2015.

Completed the requirements for the Bachelor of Science in Soil and Crop Sciences and Horticulture at Colorado State University, Ft. Collins, Colorado in 2013.

Experience:

Ph.D. Student in Entomology at OSU (January 2016- Present)
Research in Utilizing stratified sampling data to develop a scouting plan for sugarcane aphid, *Melanaphis sacchari* Zehntner in Sorghum.
USDA National Needs Fellow, Dept. of Entomology and Plant Pathology
Oklahoma State University (January 2014- December 2015)
Research in population dynamics of *Puccinia emaculata* in natural and experimental environments.
USDA APHIS CPHST Intern, Miami Florida (May 2015- July 2015)
Interception and Iradiation protocols for *Brevipalpus* mites, vectors of CiLV, biosafety protocols.