EVALUATION OF COMMERCIAL GRAIN SORGHUM HYBRIDS AND COMMERCIAL INSECTICIDES FOR CONTROL OF THE SUGARCANE APHID *Melanaphis sacchari* (ZEHNTNER, 1897) (HEMIPTERA: APHIDIDAE)

A Thesis

by

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ABSTRACT

Since the sugarcane aphid's (SCA) introduction in the United States in 2013 many management strategies have been evaluated and used for its control. In this study we evaluated hybrid resistance through a field screening, seedling screen, and reproduction study. For chemical control we found it necessary to develop baseline susceptibility data for two main insecticides used to control the SCA (Sulfoxaflor and Flupyradifurone). We also evaluated the residual activity of these insecticides with the addition of chlorpyrifos.

Results from the hybrid field screen revealed ATx2752/RTx2783, R84353, R9813, SP6929, SP73B12, SP7715, SPX760, SP78M30, P-83P17, W-844-E, and DKS 37-07 exhibited resistance, showing no statistical differences in yield or cumulative aphid days between sprayed and non-sprayed plots. The seedling screen showed similar results to the field screen with most of the same hybrids showing tolerance to SCA colonization at a highly susceptible stage; however P-83P17 appeared to be susceptible to aphid colonization at the seedling stage. Results from the reproduction study showed that DKS 37-07 was the most resistant hybrid of the hybrids evaluated, with reduced intrinsic rate of increase compared to the other hybrids. DGM75GB39 appeared to be the most susceptible hybrid in all three evaluations.

In the baseline susceptibility study the SCA appeared to be highly susceptible to both sulfoxaflor and flupyradifurone from 2014-2017, with slight shifts in susceptibility appearing due to environmental conditions and other factors besides potential resistance.

In the residual activity study all three insecticides appeared to offer very good initial kill, however flupyradifurone offered the longest residual control providing approximately 10 days in 2015 and 7 days in 2016. Sulfoxaflor was similar in initial kill, however it only provided approximately 5 days in 2015 and 2 days in 2016. Chlorpyrifos was similar to sulfoxaflor providing approximately 5 days in 2015 and 3 days in 2016.

DEDICATION

This thesis is dedicated to the most important people in my life, to my son James Deacon Gonzales; I know you came along later in this process, but having you here has given my life more meaning and purpose. I hope I can guide you through life like you have guided me since the day you were born. I will strive to be the best dad and set the best example I can for you. Shelby Dene, thank you for your love, support and encouragement during the challenges of work and graduate school. You have put up with many late nights in the field and at the office; the things you have done to keep this family going will forever be appreciated. I am truly blessed to have you in my life, I love you so much! To my sisters, Krysie and Adryane, I have always strived to set an excellent example for y'all, having you two look up to me has motivated me to be the best I could so you could have a positive role model in your life, bubba loves you both very much. Last but not least, I would like to dedicate this thesis to my parents, Lori Nino and Johnny Gonzales, thank you both for your constant love and support throughout my years in school. You have always stressed the importance of education to me, and have instilled in me the morals and ethics that I go by today. I can only hope to carry those qualities over to my own kids.

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Contributors

This work was supervised by a thesis committee consisting of Dr. David Kerns [Chair of Committee] and Dr. Greg Sword [Co-Chair] of the Department of Entomology and Dr. Ronnie Schnell of the Department of Soil and Crop Sciences.

All work conducted for the thesis was completed by the student independently.

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CHAPTER I

INTRODUCTION

In the United States, grain sorghum has traditionally been used for livestock feed (Smith 2000) and for ethanol production in a growing number of ethanol plants. (Zhang et al. 2017) According to the National Agricultural Statistics Service (NASS) an estimated 2.4 million hectares was planted to grain sorghum in the U.S. in 2018, up 7 percent from last year. Kansas and Texas, the leading sorghum-producing States, account for 74 percent of the U.S. acreage. Acres planted to sorghum in the Delta region continue to decrease. Arkansas planted area is estimated to be the second lowest on record, a record low is estimated in Mississippi, and Louisiana planted area is the lowest since 1962. Growers expect to harvest 5.29 million acres for grain, up 5 percent from last year (NASS 2018). According to the Census of Agriculture in 2012, the U.S. sorghum industry had sales of $1.8 Billion, accounting for 0.4 percent of total U.S. agriculture sales according to the 2012 census of agriculture. Although many states produce and sell sorghum, Texas and Kansas together accounted for 75 percent of U.S. sorghum sales. Texas led the country in sorghum for grain production, producing 2.6 billion kg's of grain in 2012, followed by Kansas and Louisiana producing 1.9 billion and 283.5 million kg of grain respectively (Census of Agriculture 2012). Sorghum is also gaining popularity in food products in the U.S. because of its gluten free and non-GMO properties; it is an excellent substitute for wheat, rye and barley for those who cannot tolerate gluten (Delserone 2008). In addition to food and feed uses, grain

sorghum and sorghum-sudangrass hybrids have been beneficial as a rotation crop. Root-knot nematodes and weed pests have been frequent targets of management by crop rotation, and traditional crops such as cotton, soybean and grain sorghum have been useful in rotation sequences for suppressing root-knot nematodes and weeds (McSorley et al. 1994, Clark 2007).

The sugarcane aphid [*Melanaphis sacchari* (Zehntner, 1897) (Hemiptera: Aphididae)] (SCA) is somewhat cosmopolitan, distributed throughout tropical and subtropical regions of the world on hosts in the genera including *Pennisetum* spp, *Saccharum*, spp. and *Sorghum* spp. (Blackman and Eastop 2000). The SCA was first reported in North America on sugarcane in Florida in 1977 (Mead 1978) and Louisiana in 1999 (White et al. 2001). Denmark (1988) first reported the SCA feeding on sorghum in Florida, but it was not considered an economic pest of the crop. The SCA is considered to be an economically important pest of sorghum in the countries of China (Wang 1961), Taiwan (Chang 1981a), Japan (Setokuchi 1973), India (Young 1970), South Africa (van Rensburg 1973a), and most recently in North America (Villanueva et al. 2014). Prior to 2013 in North America, population densities of SCA attacking sugarcane were considered low and their economic impact in the region was unclear (White 2001). However in the past few years in both the Southern U. S. and northern Mexico the overall occurrence of the SCA has rapidly increased, and has become an economically important insect pest, attacking various cultivated varieties and types within the genus *Sorghum*. "While previously known from the United States, its expansion into sorghum was an unexpected and significant event (Bowling et al. 2016).

The SCA was first detected in sorghum along the Texas Gulf Coast and Louisiana in 2013, where abundant populations caused significant yield losses due to poor plant vigor and head emergence, and abundant honeydew affecting harvest efficiency (Villanueva et al. 2014). Later in 2013, the aphid was also detected in selected counties in, Oklahoma, one county in Mississippi, and three northeastern states of Mexico. By the end of 2015, the aphid was reported on grain sorghum, sorghum–sudangrass hybrids, sweet sorghum, some millet varieties, and Johnsongrass in 17 states and over 400 counties in the United States and in all sorghum-producing regions in Mexico (Bowling et al. 2015). The aphid's high reproductive rate on susceptible sorghum hybrids has resulted in reports of yield loss ranging from 10% to greater than 50% (Bowling et al. 2016). The U.S. produces large yields within less arable space, and exports approximately 3.4 billion kg's of grain sorghum annually (U.S. Grains Council 2016), any yield loss resulting from SCA could have global impact.

Feeding symptoms of the SCA are manifested as reddening or purpling of leaf tissue and result in subsequent necrosis. Severe injury to seedlings can kill plants and reduce stand early in the growing season (Singh et al. 2004). Injury to pre-boot sorghum can sterilize or delay seed head development. This becomes a problem later in the season, when heading becomes inconsistent, which often results in problems with management of other sorghum pests such as sorghum midge *Stenodiplosis sorghicola* (Coquillett) and corn earworms *Helicoverpa zea* (Boddie), when sorghum begins to flower. Injury to soft or hard dough sorghum can result in reduced test weight. High SCA numbers can also cause issues with harvest efficiency, resulting in machine failure

and further jeopardizing yield potential (Brown et al. 2015). Managing the SCA requires a fully integrated approach; landscape management, timely planting, insecticide seed treatments, effective and timely scouting and treatment decisions, insecticide choice and efficacy, biological control, and selection of resistant grain sorghum hybrids. This research project will address two IPM strategies used to manage pests in crops. Objective one was to evaluate commercial grain sorghum hybrids for their degree of tolerance/resistance to the SCA in the field and in growth chamber seedling screens; in addition, I conducted a reproduction study to analyze the SCA reproductive behavior on selected hybrids to determine if resistance was, at least in part, attributable to antibiosis. Objective two was to develop baseline susceptibility data for resistance monitoring of two commercial insecticides used to control the SCA, sulfoxaflor (Transform™ WG, Dow AgroSciences LLC, Indianapolis, IN) and flupyradifurone (Sivanto® Prime, Bayer CropScience, Research Triangle PK, NC). Objective three was to determine the residual activity of three insecticides used for SCA control, chlorpyrifos (Lorsban-4E®, Dow AgroSciences LLC, Indianapolis, IN), sulfoxaflor and flupyradifurone on grain sorghum.

Objectives:

1. Evaluation of commercial grain sorghum hybrids for resistance to the SCA.

1b. Evaluation of SCA reproductive behavior on commercial grain sorghum hybrids.

2. Develop baseline susceptibility data for sulfoxaflor and flupyradifurone.

3. Determine the residual activity of chlorpyrifos, sulfoxaflor and flupyradifurone on grain sorghum for control of the SCA.

CHAPTER II

LITERATURE REVIEW

Grain Sorghum (*Sorghum bicolor*)

Sorghum is an upright, short-day, summer annual that is a member of the Poaceae family. The grass blades are flat, stems are rigid, and there are no creeping rhizomes. Sorghum has a loose, open panicle of short, few-flowered racemes. Glumes vary in color from red or reddish brown to yellowish and are at least three quarters as long as the elliptical grain. The grain is predominately red or reddish brown (Kearney and Peebles 1969; Barkworth, 2003). Sorghums are of tropical origin (Barkworth, 2003), but have spread all over the world, with current production in many countries including Africa, Australia, China, Central and South America, India, and the North America. Sorghum will grow in low fertility, moderately acidic and highly alkaline soils, but it is best adapted to fertile, well drained soils at a pH between 6.0–6.5. Sorghum is not tolerant of frost, shade, or sustained flooding (Clark 2007; Undersander 2003). Sorghum is truly a versatile crop that can be grown as a grain, forage or sweet crop, sorghum is one of the top five cereal crops in the world. According to the United Sorghum Checkoff Program (2016) the United States is the world's largest producer of grain sorghum, having produced 217 million kg in 2016. Sorghum is traditionally grown throughout the Sorghum Belt, which runs from South Dakota to Southern Texas, primarily on dryland hectares, according to the United Sorghum Checkoff Program

(2016). In 2017, sorghum was planted on 2.3 million hectares and 165 million kg were harvested (United Sorghum Checkoff Program 2016). Kansas, Texas, Colorado, Oklahoma and South Dakota were the top five sorghum-producing states in 2017, with 1.05 million hectares planted in Kansas and 120,000 hectares planted in South Dakota, according to United Sorghum Checkoff Program (2016).

Sugarcane Aphid (*Melanaphis sacchari*)

SCA's are tan, yellow, or grey in appearance with paired, darkened cornicles and tarsi (Villanueva et al. 2014). They are plant sap (phloem) feeders, which feed on the underside of leaves and along the stalk (Bowling et al. 2016). They typically colonize the lower portions of leaves first, and then move outwards and upwards as the population increases (Villanueva et al., 2014). Like other aphids, they can quickly multiply under optimal conditions, excreting large amounts of honeydew on leaf surfaces that may encourage mold growth. SCA feeding may also cause damage ranging from leaf yellowing to grain formation prevention, or even plant death (Bowling et al. 2016; Colares et al. 2015b; Villanueva et al. 2014). The SCA is an anholocyclic, parthenogenic, viviparous species, which means that it feeds on its annual hosts (*Sorghum* species) only in the spring and summer, and the same hosts that persist through the fall and winter months (Johnsongrass but also remnant grain sorghum) (Bowling 2016). All aphids are female and produce live young asexually in North America, with the exception of one report of egg production from female aphids

collected from three Mexican states (Pena-Martinez et al. 2016). Depending on nutrition and environmental conditions, adults live up to 37 days and have a reproductive potential of 34-96 nymphs per female (Singh et al., 2004).

Insecticides

Chlorpyrifos

Chlorpyrifos (Lorsban-4E®, Corteva AgriSciences LLC, Indianapolis, IN) is a broad-spectrum, chlorinated organophosphate insecticide, acaricide and nematicide. Chlorpyrifos was first registered for use in the United States in 1965 (NPIC). Chlorpyrifos is a colorless to white crystalline and has a mild mercaptan (thiol) odor, similar to the smell of sulfur compounds found in rotten eggs, onions, garlic and skunks Chlorpyrifos is a broad-spectrum insecticide which kills insects upon contact by affecting the normal function of the nervous system (NPIC) It affects the nervous system by inhibiting the breakdown of acetylcholine (ACh), a neurotransmitter, when insects are exposed, chlorpyrifos binds to the active site of the cholinesterase (ChE) enzyme, which prevents breakdown of ACh in the synaptic cleft (NPIC). The resulting accumulations of ACh in the synaptic cleft causes overstimulation of the neuronal cells, which leads to neurotoxicity and eventual death (NPIC) Chlorpyrifos is used on agricultural food and feed crops, is one of the most widely used active ingredients in insecticides in the world. Since it was first registered in the U. S. in 1965, chlorpyrifos has played a key role in pest management efforts in the U.S. and around the world.

Today, chlorpyrifos is registered in almost 100 countries worldwide for use on more than 50 different crops against damage caused by a wide range of insect pests, including the SCA. Chlorpyrifos is a critical tool for growers who rely on it because of its efficacy, low cost, tank mix compatibility, ease of implementation into existing Integrated Pest Management and Integrated Resistance Management programs.

Sulfoxaflor

The sulfoximines, as exemplified by sulfoxaflor (Transform™ WG, Corteva AgriSciences LLC, Indianapolis, IN) represent a new class of insecticides. Sulfoxaflor exhibits a high degree of efficacy against a wide range of sap-feeding insects, including those resistant to neonicotinoids and other insecticides (Sparks et al. 2013). Resistance to existing insecticides like neonicotinoids is an on-going problem that requires the development of new classes of insecticides. Numerous sap-feeding insects like the SCA including; *Myzus persicae* (green peach aphid), *Aphis gossypii* (cotton aphid), *Bemisia tabaci* (sweet potato whitefly) and *Nilaparvata lugens* (brown plant hopper), have history of developing resistance to commercially available insecticides (Whalon 2008). Because the sulfoximines and neonicotinoids both function as nAChR agonists, it might be assumed that the SARs and interactions with the insect nAChR of the two chemistries are quite similar. However, the sulfoximines and neonicotinoids are distinct just as other classes of structurally similar insecticides are distinct (Sparks et al. 2013).

Flupyradifurone

The discovery of flupyradifurone (Sivanto® Prime, Bayer CropScience, Research Triangle PK, NC) was inspired by the buenolide scaffold in naturally occurring stemofoline (Nauen et al. 2014). Sivanto acts reversibly as an agonist on insect nAChR but is structurally different from known agonists, as shown by chemical similarity analysis. It shows a fast action on a broad range of sucking pests, as demonstrated in laboratory bioassays, and exhibits excellent field efficacy on a number of crops with different application methods, including foliar, soil, seed treatment and drip irrigation (Nauen et al. 2014). In order to diversify the toolbox necessary for appropriate resistance management measures alternating with established compounds, new chemical classes of insecticides need to be introduced (McCaffery 2006). The development and commercialization of new chemical classes of insecticides for efficient crop protection measures against destructive invertebrate pests is of utmost importance to overcome resistance issues and to secure sustainable crop yields (Nauen et al. 2014). The novel butenolide insecticide flupyradifurone shows unique properties and will become a new tool for ingtegrated pest management around the globe, as demonstrated by its insecticidal, exotoxicological and safety profile (Nauen et al 2014).

CHAPTER III

EVALUATION OF COMMERCIAL GRAIN SORGHUM HYBRIDS FOR RESISTANCE TO THE SCA

Introduction

The sugarcane aphid (SCA) Melanaphis sacchari (Zehntner) (Hemiptera: Aphididae) was first discovered on sorghum [Sorghum bicolor (L.) Moench] in the United States in Florida as early as in 1922 (Wilbrink 1922), and later confirmed by Denmark (1988). However, this aphid has historically been observed infesting sugarcane, Saccharum spp. hybrids, in Florida (Mead 1978; Denmark 1988) and Louisiana (Hall 1987; White et al. 2001). In 2013, the SCA was detected in sorghum along the Texas and Louisiana in the Gulf Coast region, where abundant populations caused significant yield losses due to reduced plant vigor, head emergence, and abundant honeydew accumulation which affected harvest efficiency (Villanueva et al. 2014). Later in 2013, the aphid was also detected in selected parishes and counties in Louisiana, Oklahoma, one county in Mississippi, and three northeastern states of Mexico (Bowling et al., 2016).

By the end of 2015, the aphid was reported on grain sorghum in 17 states and over 400 counties in the United States and all sorghum-producing regions in Mexico (Bowling et al. 2015). In response to the emergence of the SCA as a severe sorghum pest, a combination projects such as, sorghum hybrid screening for host plant resistance

have been undertaken (Bowling et al. 2016). Genetic resistance in field crops to insect pests is an attractive aphid pest management tactic because of its ease of use and potential affordability and compatibility with natural enemies (Brewer and Elliott 2004). There has been substantial use of grain sorghum hybrids with resistant traits to greenbug, Schizaphis graminum (Rondani), in North America (Michels and Burd 2007). Research has begun to evaluate parental lines and commercially available grain sorghum hybrids for resistance to SCA in North America, adding to existing international efforts (Singh et al. 2004). Recently, sorghum parental types SC110 and SC170, Tx2783, and Texas A&M sorghum lines and hybrids Tx2783, Tx3408, Tx3409, B11070, B11070, AB11055-WF1-CS1/RTx436, and AB11055-WF1 CS1/RTx437 have shown high levels of resistance to SCA in greenhouse and field tests (Armstrong et al. 2015; Mbulwe et al. 2015). If resistance or tolerance is detected in current commercial hybrids, this may assist breeders in the development of newer, widely adapted hybrids with some degree of resistance to the SCA (Brown et al., 2016). Additionally, utilizing these SCA resistant hybrids, where agronomically acceptable, will likely reduce the need for insecticide applications, helping preserve natural enemies and increase producer profitability. The objective of this study was to determine the degree of resistance/tolerance of commercially available grain sorghum hybrids to the SCA through three studies; a field evaluation of 16 grain sorghum hybrids, a seedling susceptibility screen, and a reproduction study to evaluate SCA reproductive potential on hybrids with differing degrees of resistance/tolerance.

Materials and Methods

Hybrid Screen

Grain sorghum hybrids with herbicide safener (Concep II, Syngenta Crop Protection, Inc., Greensboro, NC) seed treatments were planted in May to early June, at the Macon Ridge Research Station (Winnsboro, LA), Dean Lee Research Station (Alexandria, LA), and Northeast Research Station (St. Joseph, LA). Each of these locations provides different soil types and environments, which allowed for a more fitting evaluation of resistance and yield potential.

At each location, a field experiment was conducted in 2015 and 2016. The experiments were planted using a 4-row cone planter with a seeding rate of 30,350 seeds per hectare. Depending on the location, plots were dryland or irrigated as needed using furrow irrigation. The field evaluation followed a split-plot randomized complete block design with 4 replicates. Hybrids were randomized to main plots, which were 12.2-15.2 m in length and 8 rows wide with a 0.97-1.01-m row spacing. Sub-plots were 4 rows and consisted of an insecticide-treated or a non-treated half main plot (sprayed vs. non-sprayed). A positive and a negative check were included. The positive check was a known resistant/tolerant hybrid, Tx2783 and the negative check was a known susceptible hybrid, Tx430 (Armstrong, 2015). The other hybrids included Dyna-Gro M75GB39, M77GB52, 765B (Crop Production Services, Loveland, Co), Pioneer 83P17, (Pioneer Hi-Bred International Inc., Johnston, IA), DeKalb DKS37-07, (Monsanto Company, St.

Louis, MO), Warner W-844-E, (Warner Seeds Inc., Hereford, TX), Sorghum Partners SP6929, SPX760, SP7715, SP78M30, SP73B12, (Global Sorghum Solutions, LLC., Lubbock TX), Richardson Seeds RS260E, RS94153, RS84353, (Richardson Seeds Inc., Vega, TX). The treated sub-plots were sprayed with sulfoxaflor (Transform® WG, Corteva AgriScience, Indianapolis, IN) at 0.05 kg [AI] /ha or flupyradifurone (Sivanto™ Prime, Bayer CropScience, Research Triangle Park, NC) at 0.07 kg [AI]/ha to prevent significant aphid infestation. The threshold for triggering an aphid spray was based on the first detection of colonizing SCA. All other economically important insects were controlled as needed.

Plots were monitored weekly for aphid colonization. Once colonization was detected, data were collected weekly for the duration of the infestation. Aphid infestations were estimated in each sub-plot by counting the number of aphids from 10 upper canopy and 10 lower canopy leaves. The upper canopy leaves were collected one node below the uppermost emerged leaf, while the lower canopy leaves were taken arbitrarily from the middle to lower canopy portion of the plant (Kerns et al. 2015). Aphid counts from upper and lower canopy leaves were pooled and cumulative aphid days (CAD) were calculated for each sub-plot (Ruppel et al. 1993). CAD calculations were utilized because it's a representative value of aphid density over time (Kerns et al. 2015). Once physiological maturity was reached, the entire test area was treated with glyphosate to facilitate desiccation and increase harvest efficiency. The middle two rows of each split plot were harvested using a 2-row plot combine at target 15-17% moisture. Yield was adjusted to 14% moisture.

All data were analyzed using linear mixed models in PROC GLIMMIX (SAS Institute Inc. 2011). Data across test locations were analyzed separately for each year using hybrid, insecticidal protection, and the associated two-way interaction as fixed effects. The random effects were location, block (location) and hybrid*block(location). When significant interactions ($\alpha = 0.05$) were detected between hybrid and insecticidal protection, the SLICEDIFF option of the LSMEANS statement was utilized to determine if insecticidal protection was associated with a difference in CAD and yield for each hybrid.

Seedling Screen

A seedling screen was conducted in a growth chamber to complement the field trials. Hybrids were planted in 20.3 cm pots, with five seedlings per pot. Pots were kept in a plant growth chamber (Percival Scientific, Perry IA) at 27° C ± 1° C with 70% relative humidity and a 12:12 L:D photoperiod. When seedlings reached the 2-3 leaf stage, plants were manually infested with approximately 50 aphids per plant. Each plant was rated on a 1-9 injury scale where 1= no injury and 9 = dead plant (Webster & Starks 1984), at approximately 2 weeks after infestation, or sooner depending on the visual appearance of the susceptible check. Data were analyzed using PROC GLIMMIX (PROC GLIMMIX SAS Institute Inc. 2011), using the random effect of hybrid*rep. A one-way analysis of variance (ANOVA) with the hybrid set as the treatment was

conducted, and means were separated using Tukey's honestly significant difference (HSD) test at $\alpha=0.05$.

Results

Hybrid Screen

In 2015, SCA colonized all sorghum hybrids. Differences in CAD were detected among sprayed and non-sprayed plots across all hybrids ($F = 2.89$; df = 15,161.3; $P = 0.0005$). However, a hybrid by insecticide interaction was detected ($F = 2.54$; df = 15,172; $P = 0.0020$), indicating that the effect of insecticide applications changed with hybrid (Figure 1). Differences in CAD were detected for ATx2752/RTx430 ($F = 5.59$; df = 1,172; $P < 0.0001$), M75GB39 ($F = 2.95$; df = 1,172; $P = 0.0036$), 765B ($F = 1.97$; df = 1,172; $P = 0.05$), and M77GB52 ($F = 3.22$; df = 1,172; $P = 0.0015$). Differences in CAD were not detected between sprayed and non-sprayed plots for the remaining hybrids; ATx2752/RTx2783, RS84353, RS260E, SP6929, SP73B12, SP7715, SPX760, SP78M30, P83P17, W-844-E, and DKS 37-07 (Figure 1).

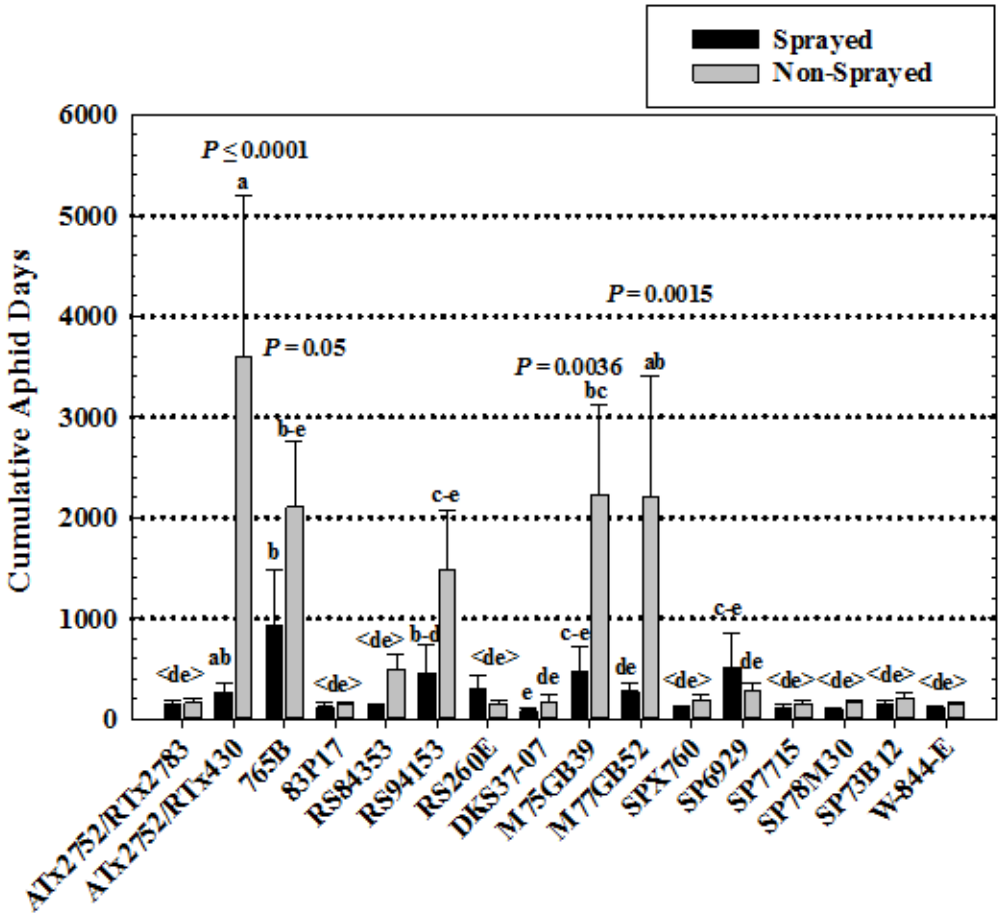

Figure III-1:CAD of 16 grain sorghum hybrids treated for SCA or left non-treated, across three locations in Louisiana in 2015. Comparisons of Hybrid*Insecticide LSM by Hybrid α = 0.05. Hybrids followed by the same letter are not statistically different α = 0.05.

In 2016, hybrids that experienced reduced CAD where insecticide applications were made include; M75GB39 ($F = 5.22$; df = 1, 87; $P \leq 0.0001$), M77GB52 ($F = 2.60$; df = 1, 87; $P = 0.0108$), 765B ($F = 2.56$; df = 1, 87; $P \leq 0.0121$), and REV 9782 ($F = 4.39$; df = 1, 87; $P \leq 0.0001$). Hybrids that experienced low CAD, in both sprayed and non-sprayed plots include; DKS37-07, 83P17, W-844-E, and SP7715 (Figure 2). We

were again able to detect a Hybrid*Insecticide interaction across all hybrids sprayed and non-sprayed (F = 13.47; df = 7, 76.24; P ≤ 0.0001) (Figure 2).

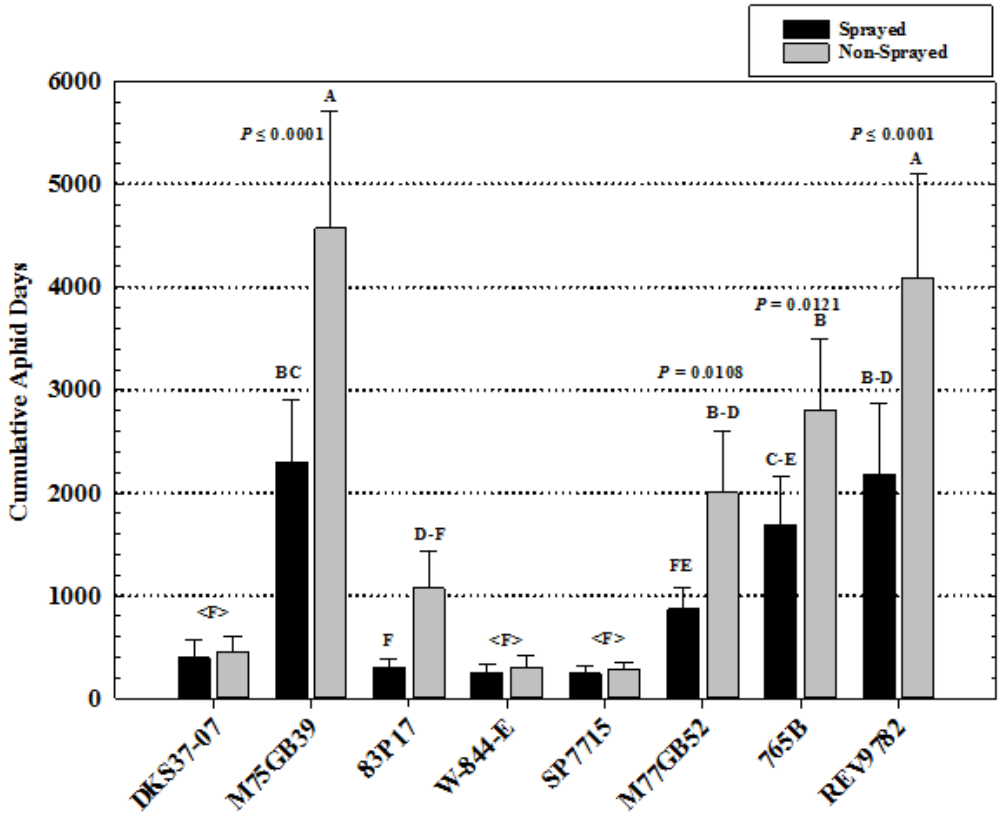

Figure III-2: CAD of 8 grain sorghum hybrids treated for SCA or left non-treated, across three locations in Louisiana in 2016. Comparisons of Hybrid*Insecticide LSM by Hybrid α = 0.05. Hybrids followed by the same letter are not statistically different α = 0.05.

Yield data for 2015 revealed increased yields for two hybrids where insecticide applications were made (Figure 3), ATx2752/RTx430 (F = 2.26; df = 1,172, P = 0.0253) and M75GB39 (F = 2.03; df = 1,172; P = 0.0438). Hybrids 765B and M77GB52 showed a decrease in CAD where applications were made but did not show increased yields. Hybrids that appeared to offer resistance, did not show increased yields where

insecticide applications were made, these hybrids include; ATx2752/RTx2783, RS84353, RS260E, SP6929, SP73B12, SP7715, SPX760, SP78M30, 83P17, W-844-E, and DKS37-07. Although we were unable to detect a Hybrid*Insecticide interaction, significant yield differences were detected across all hybrids regardless of insecticidal control ($F = 5.29$, $df = 15,161.1$, $P \leq 0.0001$) (Figure 4).

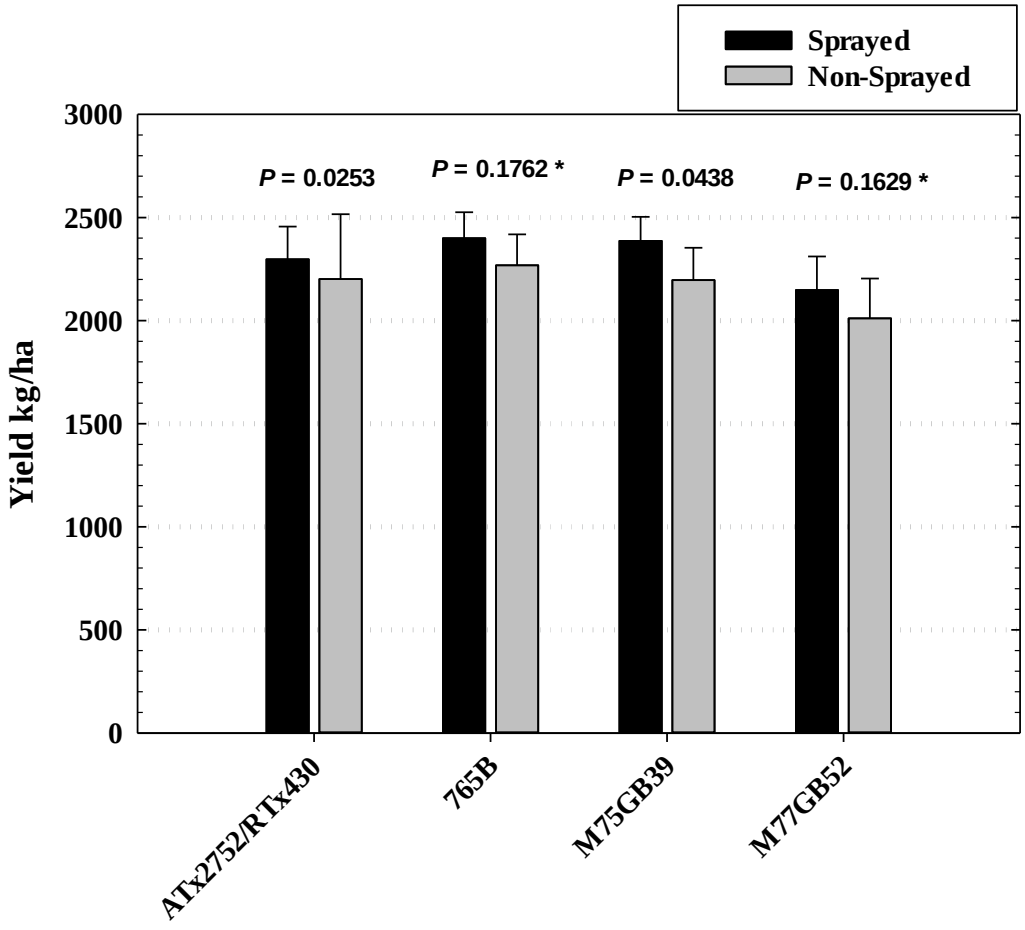

Figure III-3: Yield in kg/ha of grain sorghum hybrids that experienced increased yields where insecticide applications were made in 2015. Comparisons of Hybrid*Insecticide LSM by Hybrid $\alpha = 0.05$. * DG765B and DGM77GB52 experienced reduced CAD in sprayed plots but did not show increased yield.

In 2016, a yield benefit from insecticide applications was not detected in any of the hybrids tested at the three locations. Two locations experienced low aphid populations, while the other was jeopardized by late sampling. At the Dean Lee Research Station in Alexandria, LA, pre-counts were taken when aphid numbers had reached greater than 2,000 aphids per leaf in the susceptible hybrids, and several hundred aphids per leaf in hybrids that appeared to offer resistance in previous evaluations, so we were unable to detect a hybrid by insecticide interaction. However, differences were detected among all hybrids regardless of insecticidal application ($F = 8.19$, $df = 7, 70.87$, $P \leq 0.0001$) (Figure 5).

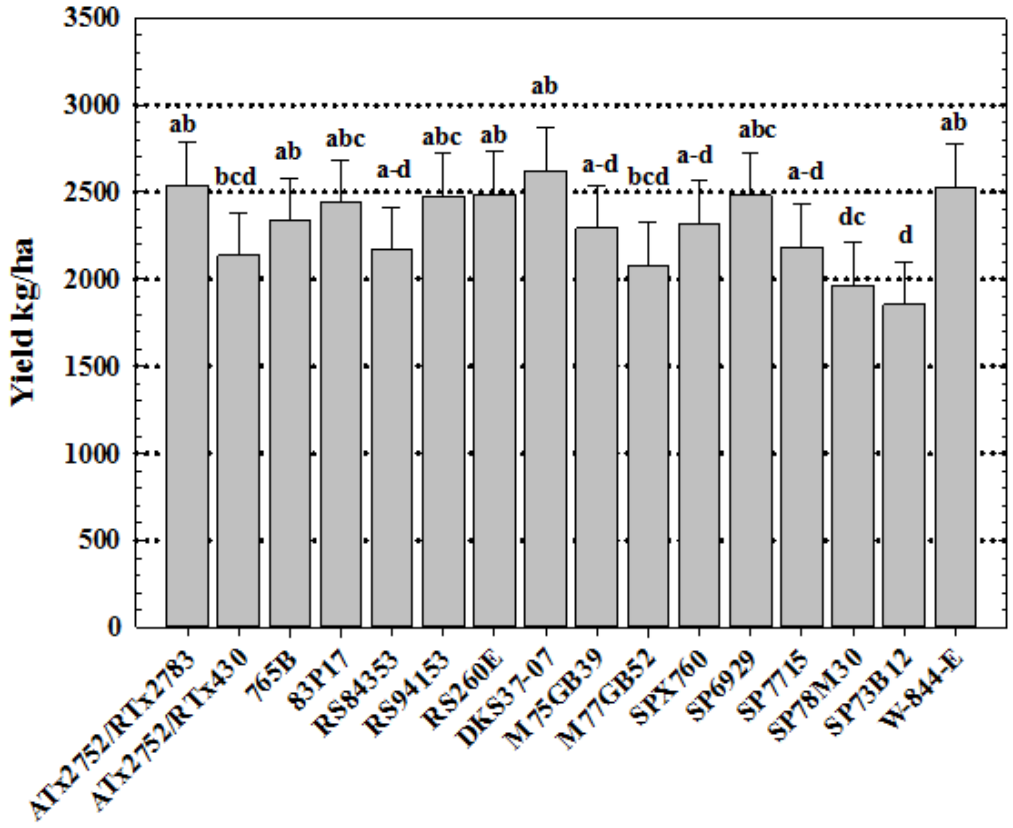

Figure III-4: Yield in kg/ha of 16 grain sorghum hybrids across three locations in Louisiana in 2015. Hybrids followed by the same letter are not statistically different α = 0.05.

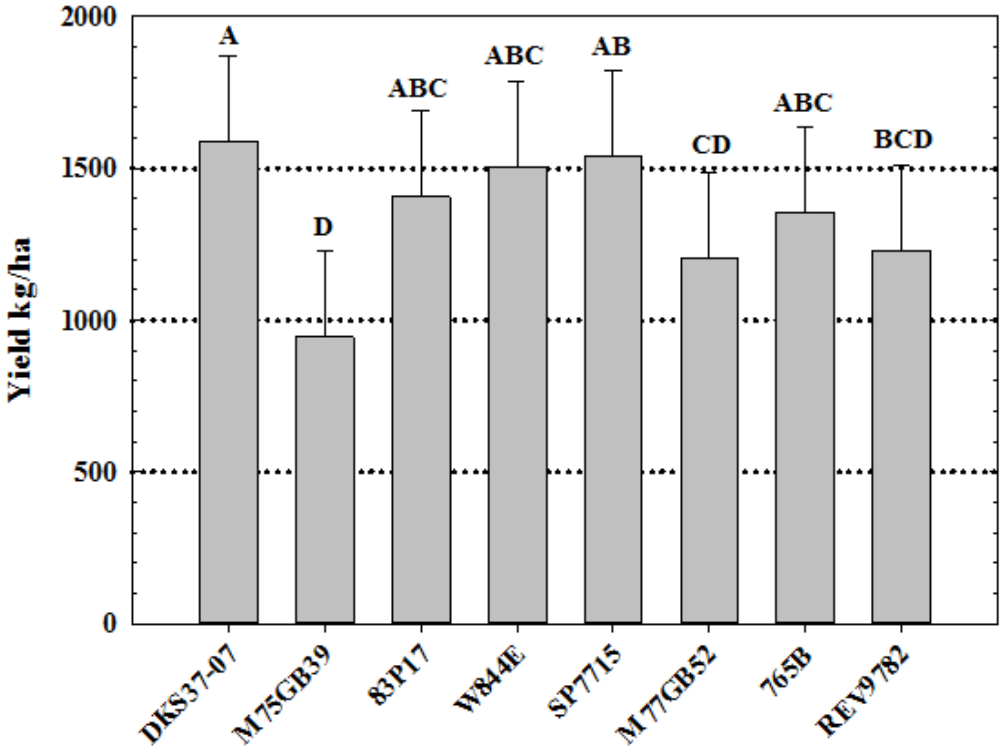

Figure III-5: Yield in kg/ha of 8 grain sorghum hybrids at the Dean Lee Research Station in 2016. Hybrids followed by the same letter are not statistically different $\alpha = 0.05$.

Seedling Screen

There were hybrids that showed significantly less feeding injury than the susceptible hybrids. These hybrids include; ATx2752/RTx2783, DKS37-07, R9813, R84353, SP7715, SP73B12, SPX760, SP78M30 and W-844-E (Figure 6). There were hybrids that appeared to offer resistance in the field evaluation and the seedling screening. These hybrids include: ATx2752/RTx2783, RS84353, RS9813, SP6929, SP73B12, SP7715, SPX760, SP78M30, W-844-E, and DKS37-07.

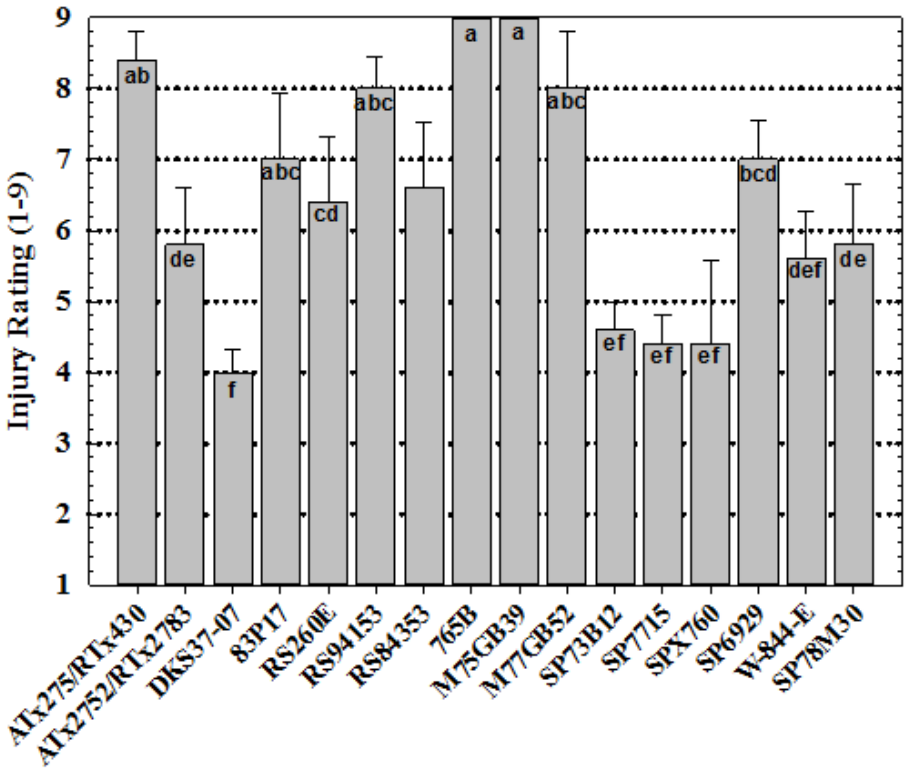

Figure III-6: Seedling Injury Screen: 1=dead plant 9= healthy plant. Hybrids followed by the same letter are not statistically different using an F protected Tukey's HSD, α = 0.05.

Discussion

Based on the hybrids used to evaluate field resistance to the SCA, we determined that there are commercially available hybrids that offer resistance or tolerance to the SCA, showing a reduction in CAD, reduced aphid colonization and no reduction in yield between sprayed and non-sprayed plots. In a study conducted by Armstrong et al. (2015), RTx2783 was proven to be a good source of resistance to the SCA based on no-choice tests in the greenhouse, and from phenotyping in the field. We evaluated

RTx2783 in our study as a known resistant hybrid. Based on our evaluations of all hybrids in the field and seedling screen, DKS 37-07 compares to RTx2783, as it offered the most resistance of any hybrid; showing no differences in yield or CAD between sprayed and non-sprayed plots, and less feeding injury in the seedling screen.

In a study conducted by Manthe (1992), 16 sorghum lines were screened for field resistance to SCA by scoring aphid abundance using an approach comparable to our study. TAM428 (resistant check) was resistant to natural infestation by the SCA (Manthe 1992). Similarly, we utilized the calculation of CAD to determine aphid abundance over a period of time. DKS37-07 and a number of other hybrids also offer a degree of resistance/tolerance to the SCA, including; RS84353, RS9813, SP6929, SP73B12, SP7715, SPX760, SP78M30 and W-844-E. The hybrids, 765B and M77GB52, showed a decrease in CAD where insecticide applications were made, but did not show increased yields. This suggests that these hybrids may be able to tolerate high aphid numbers without suffering significant yield reduction. 83P17 appeared to offer resistance in the field evaluation, showing no differences in CAD and yield regardless of an insecticide application; however, in the seedling screening 83P17 appeared to be highly susceptible to SCA feeding injury at this immature growth stage, showing no differences from the known susceptible hybrid ATx275/RTx430.

In Manthe's study from 1992, the known susceptible check, Segaolane, had a higher aphid infestation compared to the resistant check TAM428. Similarly, hybrids ATx2752/RTx430 (susceptible check) and M75GB39 appeared to be the most susceptible in both evaluations, both benefiting in a reduction of CAD and increased

yields where insecticide applications were made, and suffering significantly higher injury from SCA feeding in the seedling screen compared to the resistant check RTx2783.

Eruptive population dynamics of the SCA have proven challenging to suppression of this pest because SCA's reach exponential growth stage rapidly (Szczepaniec 2018a.). Therefore, although hybrids may offer resistance or tolerance to the SCA, insecticide applications may still be warranted. Timely insecticide applications are key to managing the SCA and mitigating their impact on sorghum yield (Szczepaniec 2018a.). It will remain important to monitor SCA populations frequently regardless of hybrid or seed treatment. Sorghum fields require weekly inspection of SCA with scouting efforts intensified to twice weekly once the aphids are detected to ensure timely applications (Szczepaniec 2018b.). Therefore, selecting a resistant hybrid is only one tool used for SCA suppression. Accompanying that hybrid with an insecticide seed treatment and intensified scouting efforts will provide growers added protection before insecticide applications are warranted.

CHAPTER IV

EVALUATION OF SCA REPRODUCTIVE POTENTIAL ON COMMERCIAL GRAIN SORGHUM HYBRIDS

Introduction

The sugarcane aphid (SCA) *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) was first discovered on sorghum [Sorghum bicolor (L.) Moench], in the United States in Florida as early 1922 (Wilbrink 1922), and later confirmed by Denmark (1988), although it also has a history of infesting sugarcane, Saccharum officinarum (L.), in Florida (Mead 1978, Denmark 1988) and Louisiana (Hall 1987, White et al. 2001). More recent, the SCA was detected in sorghum along the Texas and Louisiana Coast in 2013, where abundant populations caused significant yield losses due to reduced plant vigor, head emergence, and abundant honeydew accumulation which affected harvest efficiency (Villanueva et al. 2014). Later in 2013, the aphid was also detected in selected parishes and counties in Louisiana, Oklahoma, one county in Mississippi, and three northeastern states of Mexico (Bowling et al. 2016).

By the end of 2015, the aphid was reported on grain sorghum in 17 states and over 400 counties in the United States and all sorghum-producing regions in Mexico (Bowling et al. 2015). In response to the emergence of the SCA, a combination of research-based data such as, sorghum hybrid screening for host plant resistance have been undertaken (Bowling et al. 2016). Genetic resistance in field crops to insect pests

is an attractive aphid pest management tactic because of its ease of use and potential affordability and compatibility with natural enemies (Brewer and Elliott 2004). There has been substantial use of grain sorghum hybrids, with resistant traits to greenbug, *Schizaphis graminum* (Rondani), in North America (Michels and Burd 2007).

The SCA has four nymphal sadia (non-winged nymphs). It takes about 4-12 d for development from birth to adult, depending on temperature (Chang et al 1982). Adult longevity ranges from 10-37 d (Chang et al. 1982, Singh et al. 2004); this may be in the apterous or alate form, with a reproductive potential ranging from 34 to 96 nymphs per female depending on temperature and nutrition (Chang et al. 1982, Singh et al. 2004). Plant resistance is an important component in aphid management and has been used in other crops such as sugarcane; however plant resistance to SCA is more extensively characterized in sorghum (Singh et al. 2004) than in sugarcane (Akbar et al. 2000). The most common resistance mechanism is antibiosis (Singh et al. 2004). In this study four hybrids with differing degrees of tolerance/resistance (based on field tests and the seedling screen) were used to determine if antibiosis is a mechanism of resistance in commercially available grain sorghum hybrids. If antibiosis is a mechanism of resistance, SCA feeding on those hybrids should have a lower intrinsic rate of natural increase, or shorter life span than SCA feeding on a susceptible hybrid.

Material and Methods

Life table statistics were derived from SCA's reared on sorghum maintained in the growth chamber (Percival Scientific, Perry IA) at 27º C ± 1º C with 70 % relative humidity and 12:12 L:D photoperiod, at the Macon Ridge Research Station. Aphid exclusion cages were constructed from staked 1.9 L and 3.7 L Clear Plastic Pet Round Wide Mouth Jars (Uline, Coppell, TX). The 1.9 L jar served as the bottom jar and was used to hold soil as a growth medium for the sorghum. This jar was further modified with holes approximately 2.5 cm from the top for watering purposes. The 3.7 L jar was inverted and served as the housing for the sorghum foliage and SCA. This jar was ventilated on two sides with No-Thrips Insect Screen mesh (0.18 x 0.25 mm hole opening size) (BioQuip Products, Rancho Dominquez, CA). The jars were connected, top to top, by gluing the tops of the lids together. A 2.5 cm diameter hole was cut through both lids to allow the sorghum plant to extend from the soil in the lower jar, into the containment cage (upper jar). The hole in the lids were sealed around the plant base using plastina modeling clay to prevent aphids from dispersing from the upper containment cage into the jar containing the soil.

Aphids used for this study were obtained from laboratory colony derived from a single maternal aphid maintained on M75GB39 (Crop Production Services, Loveland, Co) in a growth chamber. The sorghum isolates evaluated included: 83P17 (Pioneer Hi-Bred International Inc., Johnston, IA), DeKalb DKS 37-07, (Monsanto Company, St.

Louis, MO), Dyna-Gro M75GB39 and Warner W-844-E (Warner Seeds Inc., Hereford, TX).

Individually caged sorghum plants with four true leaves were infested with four adult SCA on the second true leaf. Each sorghum hybrid was replicated eight times. After 12 h, and viviparous birth produced approximately five nymphs, the adult aphids were removed. All nymphs remained on the sorghum plants for two days to ensure survival. Once the nymphs reached the second or third instar, all but one nymph were removed. The remaining aphid was considered the mother aphid used for isofemale lineage. Exclusion cages containing the aphid infested plants were kept in a plant growth chamber at 27°C ± 1°C with 70 % relative humidity and 12:12 L:D photoperiod. Cages were arranged in the growth chamber in a randomized complete block design. Each remaining aphid was monitored daily until death. The number of nymphs produced were recorded and removed daily using a small 10/0 camel hair paintbrush. Life history parameters collected included pre-reproductive period (birth to onset of reproduction), reproductive period (days of reproduction), fecundity (mean reproductive output of the SCA per day during the reproductive period), and longevity.

Aphid life-table statistics were calculated based on procedures outlined by Birch (1948) and DeLoach (1974). Generation time (T) in days, is the time to reach reproductive maturity. Net reproduction rate (Ro) is the total number of nymphs produced during the isofemale's lifespan. Intrinsic rate of natural increase (rm), rm = Loge(Ro)/T, is the rate at which the population increases in a generation. Finite daily rate of increase λ = anti-logerm, which is the rate of population increase per unit of time

(day). Doubling time (DT), DT = loge2/rm, is the time (days) required for the population to double. Data were analyzed using PROC GLIMMIX (PROC GLIMMIX SAS Institute Inc. 2011), using the random effect of hybrid*rep. A one-way analysis of variance (ANOVA) with the hybrid set as the treatment was conducted, and means were separated using Tukey's honestly significant difference (HSD) test at α=0.05.

Results

Birch Method

The generation time (pre reproductive period), reproductive period and longevity of the SCA did not differ among any of the hybrids evaluated (Table 1). However, differences among hybrids were detected in the net reproductive rate (R0), ($F = 6.50$; $df = 3, 27$; $P = 0.0019$) with approximately 27 fewer nymphs produced on DKS37-07 than M75GB39 and 83P17. W-844-E did not significantly differ from any of the other hybrids, producing approximately 55 nymphs during the reproductive period. Differences were also detected in fecundity ($F = 13.04$; $df = 3, 27$; $P \leq 0.0001$). DKS37-07 produced 3 fewer aphids per day compared to M75GB39, but did not differ from W-844-E which produced on average 4.62 nymphs per day during the reproductive period, which did not differ from 83P17 which produced 5.44 nymphs per day (Table 1). Differences were also detected in DT, rm and λ (Table 2). Among the hybrids tested the lowest rm value was computed on DKS37-07 and it was approximately 23 and 25% less than on 83P17 and M75GB39, respectively. W-844-E was not significantly different

from any of the other hybrids (F = 8.28; df = 3, 27; P = 0.0005). λ was approximately 9% lower on DKS37-07 than on M75GB39 and 83P17, and again not statistically different than W-844-E (F = 7.97; df = 3,27; P = 0.0006). DT on DKS37-07 was 1.3-fold greater than on M75GB39 and 83P17, and was not statistically different than W-844-E (F = 8.89; df = 3, 27; P = 0.0003).

Table IV- 1: Life history parameters with mean ± SE of SCA reared on commercial grain sorghum hybrids- antibiosis test.

Hybrid	Generation Time (days)	Reproductive Period (Days)	Net Reproductive Rate (R_0)	Longevity	Fecundity
P83P17	5.13 ± 0.13 a	14.13 ± 4.85 a	74.38 ± 1.06 a	23.38 ± 1.05 a	5.44 ± 0.39 ab
DKS 37-07	6.00 ± 0.33 a	14.63 ± 0.87 a	47.88 ± 5.70 b	22.25 ± 1.16 a	3.29 ± 0.39 c
M75GB39	5.13 ± 0.23 a	11.38 ± 0.32 a	75.00 ± 3.29 a	22.13 ± 1.04 a	6.64 ± 0.39 a
W-844-E	5.50 ± 0.19 a	12.14 ± 1.20 a	55.86 ± 7.41 ab	21.71 ± 2.15 a	4.62 ± 0.42 bc

Means within columns followed by the same letter are not significantly different ($\alpha = 0.05$; Tukey-Kramer Grouping for Hybrid LSM).

Table IV- 2: Demographic statistics with mean ± SE of SCA reared on commercial grain sorghum hybrids- antibiosis test.

Hybrid	Intrinsic Rate of Increase (r_m)	Doubling Time (Days)	Finite rate of increase (λ)
P83P17	0.366 ± 0.010 a	1.91 ± 0.06 b	1.44 ± 0.02 a
DKS 37-07	0.280 ± 0.013 b	2.51 ± 0.12 a	1.33 ± 0.02 b
M75GB39	0.371 ± 0.018 a	1.90 ± 0.07 b	1.45 ± 0.03 a
W-844-E	0.313 ± 0.018 ab	2.25 ± 0.12 ab	1.37 ± 0.03 ab

Means within columns followed by the same letter are not significantly different ($\alpha = 0.05$; Tukey-Kramer Grouping for Hybrid LSM).

Wyatt and White Method

When analyzing the same data using the methods outlined by Wyatt and White (1977), the pre-reproductive period, reproductive period and net reproductive rate were not influenced by any of these hybrids (Table 3). Differences among hybrids were detected in three parameters, DT, intrinsic rate of increase and λ as in the in previous method used. The hybrid with the lowest r_m value computed on DKS 37-07, it was approximately 20 and 21% less than on P83P17 and DGM75GB39 respectively. W844E was not significantly different than any of the hybrids (F=8.79; df=3, 28; P=0.0003). λ was 5% lower on DKS 37-07 than on DGM75GB39 and P83P17, and again not statistically different than W-844-E. (F=8.64; df=3, 28; P=0.0003) DT on DKS 37-07 was 1.3-fold greater than on DGM75GB39 and P83P17, and was not statistically different than W-844-E (F=9.96; df=3, 28; P=0.0001) (Table 4).

Table IV- 3: Life history parameters with mean ± SE of SCA reared on commercial grain sorghum hybrids- antibiosis test using the Wyatt and White 1977 method.

Hybrid	Generation Time/Pre-Reproductive Period (days)	Reproductive Period (Days)	Net Reproductive Rate (M_d)
P83P17	5.125 ± 0.125 a	5.125 ± 0.125 a	34.625 ± 2.528 a
DKS 37-07	6.000 ± 0.327 a	6.000 ± 0.327 a	27.625 ± 2.884 a
DGM75GB39	5.125 ± 0.226 a	5.125 ± 0.226 a	34.875 ± 2.709 a
W-844-E	5.500 ± 0.189 a	5.500 ± 0.189 a	31.375 ± 2.104 a

Means within columns followed by the same letter are not significantly different (*a= 0.05*; Tukey-Kramer Grouping for Hybrid LSM).

Table IV- 4: Demographic statistics with mean ± SE of SCA reared on commercial grain sorghum hybrids- antibiosis using Wyatt and White 1977 method.

Hybrid	Intrinsic Rate of Increase (r_m)	Doubling Time (Days)	λ
P83P17	0.221 ± 0.007 a	3.154 ± 0.099 b	1.248 ± 0.008 a
DKS 37-07	0.177 ± 0.005 b	3.947 ± 0.123 a	1.193 ± 0.007 b
DGM75GB39	0.223 ± 0.009 a	3.138 ± 0.119 b	1.250 ± 0.011 a
W-844-E	0.202 ± 0.008 ab	3.477 ± 0.136 ab	1.224 ± 0.010 ab

Means within columns followed by the same letter are not significantly different (*a= 0.05*; Tukey-Kramer Grouping for Hybrid LSM).

Discussion

According to Birch (1948), the intrinsic rate of increase is a basic parameter which an ecologist may wish to establish for an insect population. Birch further states that the rate of increase is defined per head under specified physical conditions, in an unlimited environment where the effects of increasing density do not need to be considered. The growth of such a population is by definition exponential according to Birch. Birch brought to question, what is the rate of increase of a newly emerged adult insect in an unlimited environment? The rate will vary with time as immature stages are produced until the population has a stable age distribution, stated Birch. In order for a species to survive in a particular environment it may need to have evolved a certain minimum value for its intrinsic rate of natural increase, if its rate of increase is less than this it may succumb in the struggle for existence, stated Birch. He further states that it does not necessarily follow that the higher the intrinsic rate of increase the more

successful the species will be. Evolution may operate to select species with an intrinsic rate of increase which is both large enough to enable them to compete successfully with other species and small enough to prevent a rate of multiplication which would exhaust the food supply in the environment (Birch 1948).

According to Wyatt and White (1977), the young produced in the first few days of reproduction contribute most to the value of r_m, the proportional contribution falling rapidly for later progeny. DeLoach (1974) calculated the reproductive time required to contribute 95% to the r_m and noted that the period was shorter when development was more rapid. Further examination reveals that the period corresponds closely in value to the pre-reproductive period according to Wyatt and White (1977). Thus if the pre-reproductive period is d, then 95% of the r_m will be achieved in about 2d, measured from birth. The effective fecundity can therefore be regarded as the number of young (M_d) produced in a reproductive period equal to d. This assumption is valid only if patterns of reproduction are similar. As stated before Wyatt and Whites (1977) claim is, reproduction rises rapidly at first, remains fairly constant up to the 95% date noted by DeLoach in (1974), then falls gradually over a variable time, unless reproduction has been very adversely affected.

Data from the reproduction study showed no differences in generation time, reproductive period and longevity. However differences were detected in intrinsic rate of increase (r_m), DT, λ, net reproductive rate (R_0) and fecundity. In a study conducted by Manthe 1992, antibiosis was investigated by monitoring aphid longevity, days in reproduction (reproductive period) and average number of nymphs per female (net

reproductive rate) on excised leaves of sorghum lines. Similar to the results reported by Manthe, in this study, there were no significant differences in averages of longevity and reproductive period on excised leaf sections. In addition, Manthe in 1992 investigated aphid longevity and reproduction on whole sorghum plants. Aphid longevity ranged from 4.2 d on TAM428 a resistant check in the study to 16.0 d on IS12661C a line similar to the susceptible check Segaolane. Reduced longevity and failure to reproduce were responsible for the high levels of antibiosis, which is the most common plant resistance mechanism to aphids (Manthe 1992). Although differences in longevity and reproductive period were not detected in this study, we were able to detect differences in net reproductive rate, fecudity and all three demographic statistics. DKS37-07 showed the lowest net reproductive rate, fecudity, rm, λ and highest doubling time of the 3 hybrids, but was not statistically different from W-844-E in these parameters. W-844-E appeared to provide moderate antibiosis relative to DKS37-07 the suspected resistant hybrid.

Using the Wyatt and White method, differences were only detected in the demographic statistics; intrinsic rate of increase (r_m), DT, and λ. When comparing the net reproductive data from the two methods, the claim made by Wyatt and White (1977) appears to be false. The SCA produced anywhere from 47-58% of their off spring in the time equal to the generation time or pre- reproductive period, which was approximately 22% of the entire lifespan (longevity) of the aphid on all of the hybrids tested using the method outlined by Birch (1948). Birch (1948) stated evolution may operate to select species with an intrinsic rate of increase which is both large enough to enable them to

compete successfully with other species and small enough to prevent a rate of multiplication which would exhaust the food supply. Since the SCA's were evaluated on hybrids with deferring degrees of resistance/tolerance, then Wyatt and Whites (1977) statement "The effective fecundity can therefore be regarded as the number of young (M_d) produced in a reproductive period equal to d. This assumption is valid only if patterns of reproduction are similar", appears to be true. Since the aphid's reproduction was affected by certain hybrids that offered differing degrees of resistance/tolerance, then patterns of reproduction in this study would not be similar.

Based on our findings we decided to take a similar approach to that of Wyatt and White (1977), and create a correcting constant that would fit all hybrids regardless of their degree of resistance/tolerance. As Wyatt and White did, we took all data sets for the SCA across all hybrids to calculate our new constant. The average r_m of the Birch method was divided by the average r_m of the Wyatt and White method, then multiplied by 0.738, which is the constant calculated by Wyatt and White. That equation looks like this:

$$X=\text{Mean } r_m(\text{Birch})/\text{Mean } r_m \text{ (W\&W)}$$

$$X=Y/0.738$$

To test this equation we took a single data set from a single hybrid and compared the new r_m back to the Birch (1948) method. We then tested this equation using the averages within each hybrid, then across all hybrids to further test the validity of it. The resulting constant values of 1.171, 1.224, 1.219, and 1.148, for DKS 37-07, DGM75GB39, P83P17 and W-844-E respectively, using this equation did not differ

significantly (*a=0.05*). Calculating this equation across all four hybrids gave us a new correction constant of 1.191, which is a constant acceptable for all four hybrids. The equation for calculating r_m using the new constant can now be written:

$$r_m=1.191(\log_e M_d)/d.$$

Using this relationship, new r_m values were calculated; data from the new r_m values and r_m values derived from the Birch and Wyatt and White methods were subject to a one-way analysis of variance with the method for calculating r_m set as the treatment. The relationship was statistically different ($P<0.0001$), using an F protected Tukey's HSD, *a= 0.05*. (Table 5)

Table IV-5: Comparison of means ± SEM using three different methods to calculate intrinsic rate of increase (r_m), of the SCA on four commercial grain sorghum hybrids

	Method for Calculating r_m			Averaged across hybrids		
Hybrid	Birch (1948)	Wyatt and White (1977)	New Method	Birch (1948)	Wyatt and White (1977)	New Method
DKS 37-07	0.280 ± 0.013 a	0.177 ± 0.005 b	0.285 ± 0.009 a			
DGM75GB39	0.371± 0.018 a	0.223 ± 0.009 b	0.360 ± 0.014 a	0.333 ± 0.010 a	0.206 ± 0.027 b	0.332 ± 0.008 a
83P17	0.366 ± 0.011 a	0.221 ± 0.007 b	0.357 ± 0.011 a			
W-844-E	0.313 ± 0.018 a	0.202 ± 0.023 b	0.325 ± 0.013 a			

Means within the same row followed by the same letter are not significantly different using an F protected Tukey's HSD, *a=0.05*.

CHAPTER V

DEVELOP BASELINE SUGARCANE APHID SUSCEPTIBILITY DATA FOR SULFOXAFLOR AND FLUPYRADIFURONE

Introduction

Since the introduction of the SCA on grain sorghum in 2013 in the Gulf of Texas and Louisiana, SCA's have been sprayed with numerous products, mainly products that have been used to control other aphid species infesting sorghum, since no products were labeled for the SCA. In 2014 sulfoxaflor (Transform™ WG, Dow AgroSciences LLC, Indianapolis, IN) received a Section 18 Emergency Use label for SCA control in sorghum. Sulfoxaflor is an agonist at insect nicotinic acetycholine receptors (nAChRs). In 2015 flupyradifurone (Sivanto® Prime, Bayer CropScience, Research Triangle PK, NC) received a Section 3 label for use in sorghum. Sulfoxaflor and flupyradifurone are both group 4 insecticides, but are placed in different subgroups (IRAC). Similar to other group 4 insecticides, flupyradifurone and sulfoxaflor are shown to address insect nicotinic acetylcholine receptors (nAChRs) in- secticides (Nauen et al. 2014). Compounds sharing a common target site, but representing very different types of chemistry (e.g. acetylcholinesterase inhibitors; carbamates [Group 1A] and organophosphates [Group 1B]) are placed in different subgroups because they can have distinctly different metabolic profiles minimizing the chances for metabolic cross-resistance (Sparks 2015) The sulfoximine (sulfoxaflor), and the butenolide

(flupyradifurone), are un- questionably nAChR agonists but structurally distinct from neonicotinoids and thus have been placed in new subgroups (4C and 4D respectively) in the IRAC classification scheme. This distinction is supported by data showing that aphids and whiteflies with metabolic resistance to imidacloprid and other conventional neonicotinoids remain almost fully susceptible to both sulfoxaflor and flupyradifurone (Bass 2015). However, a strain of M. persicae with the still geographically-restricted R81T mutation showed appreciable resistance to both of these new compounds (Bass 2015). Thus, anticipating risks of cross-resistance involving novel members of a broad mode-of-action group requires caution as these risks can be mechanism-specific (Bass 2015).

The objective of this research project was to develop baseline SCA susceptibility data to sulfoxaflor and flupyradifurone for future resistance monitoring efforts.

Methods

SCA's used for bioassays were collected from grain sorghum fields by cooperators in Alabama, Arkansas, Georgia, Louisiana, Mississippi, Oklahoma, South Carolina, Tennessee, and Texas prior to any insecticide application targeting SCA. Collection kits consisted of an Insulated Foam Cooler (40 cm tall x 57.15 cm wide), (Uline Coppell, TX), containing 5-6 large pizza boxes approximately (25.4 cm x 25.4 cm) (Donated by Foxes Pizza Den, Winnsboro, LA), and three 32 oz. (26.0 cm L x 20.3 cm W x 3.8 cm H) Gel Cold Packs (Uline, Coppell, TX). Field collected SCAs, on

sorghum leaves, were shipped over night to our laboratory at the Macon Ridge Research Station in Winnsboro, LA. All bioassays were conducted within 24 h of collection. Methods were adapted from those described by the Insecticide Resistance Action Committee (IRAC, Method No. 019). Bioassay arenas consisted of individual 29 ml Solo Portion Cups (Uline, Coppell, TX) with a 2 mm layer of 1% Agar solution (Sigma-Aldrich Co. LLC, St. Louis, MO) (9 g/500 mL water) in the bottom. A 3 cm diameter hole was cut into each lid and sealed with a piece of single ply tissue paper to allow excess moisture to escape (Figure 1).

Figure V-1: Bioassay cup depicting single ply tissue paper for ventilation.

Commercial formulations of sulfoxaflor and flupyradifurone were used to prepare a series of insecticides for bioassay. Insecticide concentrations tested included: 0.0, 0.5, 2.0, 5.0, 10.0, 15.0, 25.0, and 35.0 ppm active ingredient for each insecticide. Insecticides were diluted in water to obtain 500 ml of solution at the various concentrations. A non-ionic surfactant, Induce® (Helena Chemical Company,

Collierville, TN) was added to each solution at a rate of 0.5% v/v to ensure even distribution across the surface of the leaf disc.

Sorghum leaves were removed from the 8th node of non-treated field grown plants and washed with water, to remove any naturally occurring aphids and debris. Leaves were rinsed well and allowed to air dry. Leaves were then cut into 10 cm sections using scissors. Individual leaf sections were dipped into individual insecticide solutions and swirled for 5 seconds. Leaf sections for the non-treated treatment were dipped and swirled in water with non-ionic surfactant only. Leaf sections were then placed on paper towels with the abaxial surface facing up, and allowed to air dry. When completely dry, four 3 cm leaf discs were cut using an Osborne Arch Punch No. 149 (C.S. Osborne & Co., Harrison, NJ) from each leaf section, two from either side of the leaf midrib. Individual leaf discs were placed in bioassay cups, with the abaxial surface against the agar. A 31.75 mm outer diameter x 12.70 mm inner diameter flat steel washer (Kiper Hardware & Lumber, Winnsboro, LA) were placed over the leaf disc to prevent the leaf from curling and creating a flat, defined area for the aphids to feed (Figure 2). A small 10/0 camel hair paint brush, was used to transfer 10 SCA adults from the field-collected sorghum leaves onto the exposed treated leaf disc. A total of eight leaf discs were used for each insecticide concentration; thus each bioassay was replicated eight times.

Cups were held in a Percival Reach in Plant Growth Chamber (Percival, Perry, IA), at 27 1^{o} C and a 12:12 L:D photoperiod. After 48 h of exposure to treated leaves, mortality was scored based on the inability of aphids to show coordinated movement after being lightly prodded with a small paint brush.

Figure V-2: Bioassay arena depicting steel washer, leaf tissue and aphids.

Data was analyzed using PoloPlus Probit and Logit Analysis version 1.0 (LeOra Software, Pealuna, CA). LC_{50} values along with 95% confidence intervals were obtained for each SCA population tested. Regression correlation was performed using (SigmaPlot 12.0: User's Guide, 2010) regression analysis, comparing LC_{50} values of sulfoxaflor and flupyradifurone by location. Box and whisker plots were created using (SigmaPlot 12.0: User's Guide, 2010), to compare susceptibility of the SCA to sulfoxaflor and flupyradifurone over years.

Results

Sulfoxaflor

Between 2014 and 2016, 41 aphid populations were evaluated for susceptibility to sulfoxaflor. In 2014 LC50 values ranged from 0.58-14.77 parts per million (ppm) with

a mean of 3.64 ppm (Figure 3). The aphid population that was most susceptible was collected from Rapides Parish, LA, and the least susceptible population was collected from Franklin Parish, LA (Table 1). In 2015 LC50 values ranged from < 0.05-13.76 ppm with a mean of 4.92 ppm (Figure 4).

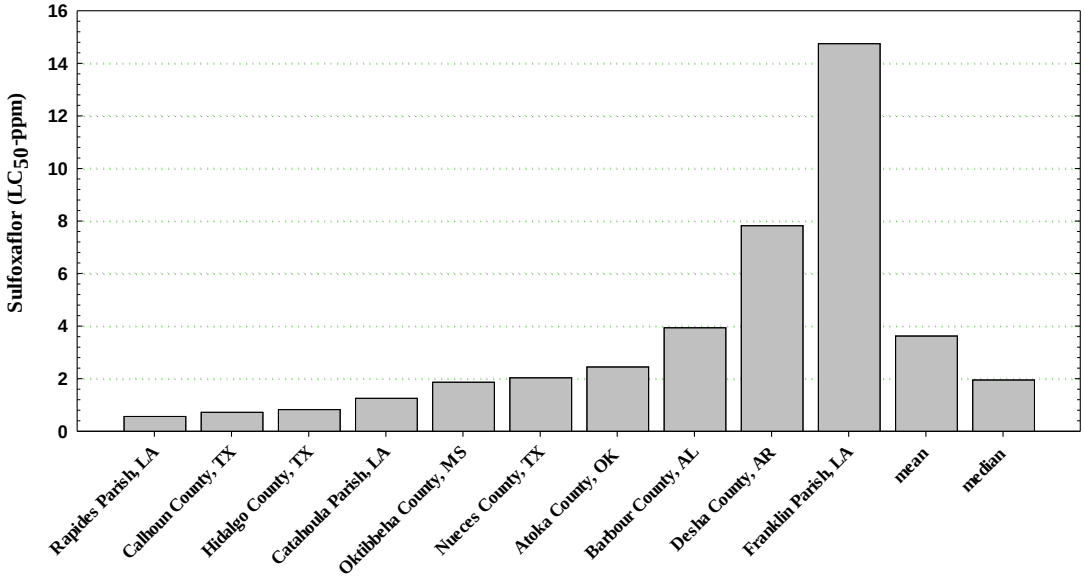

Figure V-3: 2014 Sulfoxaflor LC_{50} values by location.

The most susceptible aphid population in 2015 was collected in Castro County, TX with an LC_{50} of < 0.5 ppm; aphids collected in Franklin Parish, LA were the least susceptible, with an LC_{50} of 13.76 ppm (Table 1). In 2016 LC_{50} values ranged from 0.34-3.66 ppm with a mean of 3.64 ppm (Figure 5). The aphid population that was most susceptible was collected from Gibson County, TN, and the least susceptible population was collected from Rapides Parish, LA (Table 2).

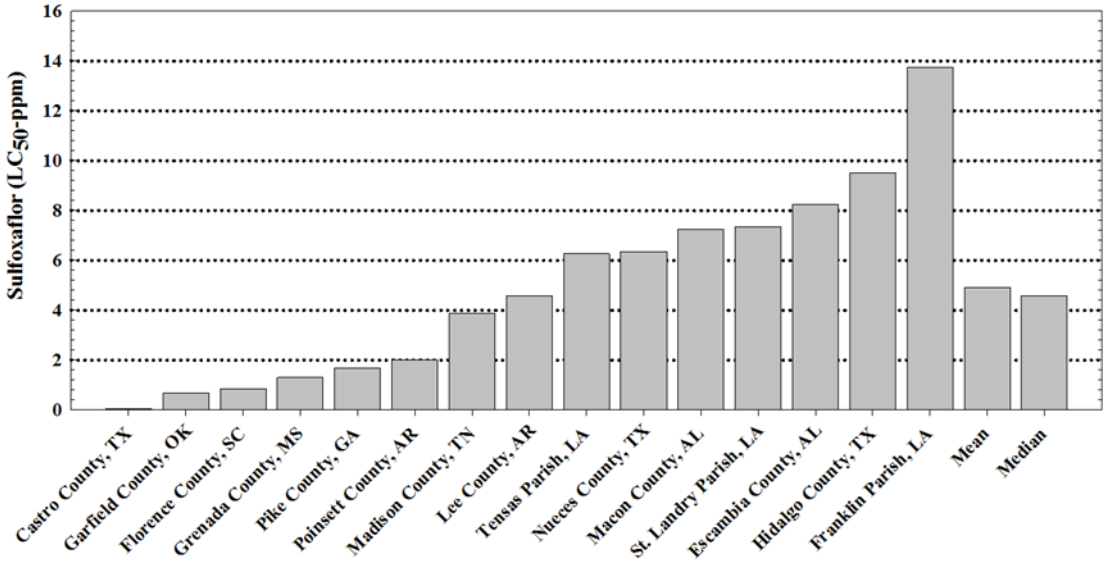

Figure V-4: 2015 Sulfoxaflor LC_{50} values by location.

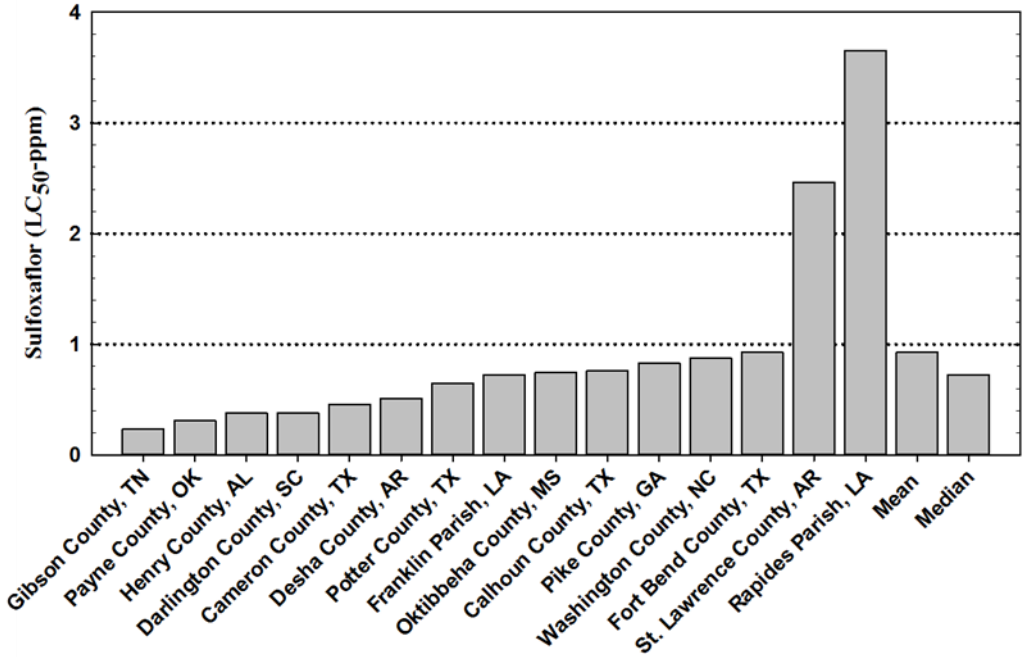

Figure V-5: 2016 Sulfoxaflor LC_{50} values by location.

Table V-1: Sulfoxaflor bioassay data sorted by month, year, and location for 2014-2015.

Population	Date (M/Y)	n	LC_{50}-ppm (95% Cl)	LC_{90}-ppm (95% Cl)
Desha County, AR	July 2014	560	7.848 (2.728-71.897)	2955.900 (194.570- x)
Rapides Parish, LA	July 2014	560	0.583 (0.450-0.738)	4.731 (3.373-7.405)
Catahoula Parish, LA	July 2014	560	1.272 (0.710-1.963)	19.720 (10.572-58.001)
Nueces County, TX	July 2014	310	2.054 (0.766-5.128)	32.218 (9.973-1721.869)
Barbour County, AL	August 2014	560	3.956 (1.858-12.095)	830.530 (117.00-x)
Franklin Parish, LA	August 2014	720	14.772 (8.899-33.251)	564.30 (158.230-6574.900)
Oktibbeha County, MS	August 2014	560	1.891 (0.844-2.632)	7.439 (5.035-22.554)
Atoka County, OK	August 2014	560	2.466 (1.045-4.404)	56.019 (20.849-742.060)
Calhoun County, TX	August 2014	560	0.741 (0.486-1.025)	12.550 (7.006-31.933)
Hidalgo County, TX	August 2014	560	0.846 (0.477-1.313)	5.127 (3.544-8.674)
Macon County, AL	June 2015	469	7.262 (4.706-9.480)	21.075 (18.269-24.986)
Tensas Parish, LA	June 2015	489	6.275 (-0.033-10.527)	41.589 (31.422-67.524)
St. Landry Parish, LA	June 2015	475	7.339 (6.182-8.454)	19.627 (17.427-22.680)
Nueces County, TX	June 2015	452	6.356 (4.616-7.937)	21.377 (18.743-25.116)
Hidalgo County, TX	June 2015	491	9.500 (6.496-12.176)	34.762 (28.792-45.221)
Escambia County, AL	July 2015	463	8.245 (6.536-9.870)	24.029 (21.263-27.875)
Poinsett County AR	July 2015	458	< 2	< 2
Grenada County, MS	July 2015	391	1.296 (-2.077-3.274)	10.624 (7.881-17.144)
Florence County, SC	July 2015	507	0.849 (-4.456-4.017)	13.571 (10.309-19.228)
Lee County, AR	August 2015	464	4.593 (2.645-6.232)	19.601 (16.662-24.151)
Franklin Parish, LA	August 2015	476	13.761 (7.012-19.070)	40.831 (33.540-52.735)
Garfield County, OK	August 2015	468	0.688 (-2.428-2.760)	15.149 (12.239-20.395)
Madison County, TN	August 2015	477	3.867 (0.974-6.055)	16.102 (13.603-19.770)
Castro County, TX	August 2015	532	< 0.5	< 0.5
Pike County, GA	October 2015	532	1.687 (0.381-2.619)	5.108 (4.138-6.571)

bSusceptibility ratio LC of population/ LC of the most susceptible population.

Table V-1 continued: Sulfoxaflor bioassay data sorted by location for 2014-2015.

Population	Slope (SE)	Chi-square, DF, Heterogeneity	SR-LC_{50}[b]	SR-LC_{90}[b]
Desha County, AR	0.498 (0.103)	98.706, 54, 1.828	33.974	2532.905
Rapides Parish, LA	1.409 (0.108)	70.940, 54, 1.314	2.524	4.054
Catahoula Parish, LA	1.077 (0.133)	40.270, 26, 1.549	5.506	16.898
Nueces County, TX	1.072 (0.182)	79.890, 28, 2.853	8.892	27.608
Barbour County, AL	0.552 (0.095)	84.844, 54, 1.571	17.126	711.680
Franklin Parish, LA	0.810 (0.122)	46.207, 34, 1.359	63.948	483.548
Oktibbeha County, MS	2.154 (0.394)	122.20, 54, 2.263	8.186	6.374
Atoka County, OK	0.945 (0.163)	103.66, 54, 1.919	10.675	48.003
Calhoun County, TX	1.526 (0.144)	92.958, 54, 1.721	3.208	10.754
Hidalgo County, TX	1.094 (0.116)	98.915, 54, 1.832	3.662	4.393
Macon County, AL	0.093 (0.009)	65.996, 54, 1.222	31.437	18.059
Tensas Parish, LA	0.036 (0.005)	123.33, 52, 2.372	27.165	35.638
St. Landry Parish, LA	0.104 (0.010)	35.870, 54, 0.664	31.771	16.818
Nueces County, TX	0.085 (0.009)	31.105, 53, 0.587	27.515	18.318
Hidalgo County, TX	0.051 (0.006)	96.791, 54, 1.792	41.126	29.787
Escambia County, AL	0.081 (0.008)	26.111, 53, 0.493	35.693	20.590
Poinsett County AR	0.036 (0.080)	264.95, 54, 4.906	*	*
Grenada County, MS	0.137 (0.017)	181.33, 46, 3.942	5.610	9.104
Florence County, SC	0.101 (0.013)	118.82, 53, 2.242	3.675	11.629
Lee County, AR	0.085 (0.009)	74.480, 53, 1.405	19.883	16.796
Franklin Parish, LA	0.047 (0.007)	73.081, 54, 1.353	59.571	34.988
Garfield County, OK	0.089 (0.012)	88.885, 53, 1.677	2.978	12.981
Madison County, TN	0.105 (0.013)	63.449, 54, 1.175	16.740	13.798
Castro County, TX	0.050 (0.021)	60.850, 54, 1.127	*	*
Pike County, GA	0.375 (0.062)	54.609, 53, 1.030	7.303	4.377

bSusceptibility ratio LC of population/ LC of the most susceptible population.

Flupyradifurone

Between 2015 and 2017, 39 aphid populations were evaluated for susceptibility to flupyradifurone. LC50 values in 2015 ranged from 0.38-15.49 ppm with a mean of 8.36 (Figure 6). The most susceptible aphid population was collected in Castro County, Texas, with an LC50 value of 0.38 ppm, while the least susceptible population was from Franklin Parish, Louisiana with a LC50 of 15.49 ppm (Table 3).

Table V-2: Sulfoxaflor bioassay data sorted by month location for 2016-2017.

Population	**Date (M/Y)**	**n**	**LC_{50}-ppm (95% Cl)**	**LC_{90}-ppm (95% Cl)**
Rapides Parish, LA	June 2016	418	3.656 (0.807-6.526)	146.331 (47.573-8341.869)
Fort Bend County, TX	June 2016	431	0.925 (0.527-1.370)	9.258 (6.629-14.363)
Calhoun County, TX	June 2016	507	0.761 (0.388-1.195)	12.166 (8.474-19.915)
St. Lawrence County, AR	July 2016	418	2.461 (1.634-3.323)	15.989 (12.047-23.239)
Cameron County, TX	July 2016	453	0.454 (0.265-0.649)	2.432 (1.824-3.489)
Franklin Parish, LA	July 2016	512	0.725 (0.366-1.149)	20.552 (13.353-38.428)
Henry County, AL	August 2016	437	0.378 (0.207-0.559)	2.196 (1.616-3.218)
Desha County, AR	August 2016	526	0.508 (0.367-0.638)	1.171 (0.903-1.898)
Pike County, GA	August 2016	463	0.830 (0.483-1.222)	7.735 (5.609-11.581)
Oktibbeha County, MS	August 2016	253	0.749 (0.408-1.127)	5.411 (3.763-8.838)
Washington County, NC	August 2016	468	0.873 (0.695-1.061)	2.624 (2.101-3.524)
Payne County, OK	August 2016	538	0.309 (0.180-0.433)	1.582 (1.216-2.251)
Darlington County, SC	August 2016	454	0.378 (0.134-0.708)	9.189 (6.186-15.634)
Gibson County, TN	August 2016	422	0.231 (0.051-0.422)	1.167 (0.738-2.052)
Potter County, TX	August 2016	477	0.648 (0.365-0.976)	6.928 (5.077-10.077)
Frio County, TX	2017	650	0.740 (0.000-3.516)	148.240 (22.607-x)

bSusceptibility ratio LC of population/ LC of the most susceptible population.

Table V-2 continued: Sulfoxaflor bioassay data sorted by location for 2016-2017.

Population	**Slope (SE)**	**Chi-square, DF, Heterogeneity**	**SR-LC_{50}[b]**	**SR-LC_{90}[b]**
Rapides Parish, LA	0.800 (0.157)	99.340, 45, 2.208	15.827	125.391
Fort Bend County, TX	1.281 (0.155)	49.475, 53, 0.933	4.004	7.933
Calhoun County, TX	1.064 (0.133)	30.129, 53, 0.568	3.294	10.425
St. Lawrence County, AR	1.577 (0.181)	32.789, 54, 0.607	10.654	13.701
Cameron County, TX	1.757 (0.237)	25.049, 54, 0.464	1.965	2.084
Franklin Parish, LA	0.882 (0.109)	33.551, 52, 0.645	3.139	17.611
Henry County, AL	0.709 (0.234)	21.145, 54, 0.392	1.636	1.882
Desha County, AR	3.538 (0.726)	12.092, 54, 0.224	2.199	1.003
Pike County, GA	1.322 (0.147)	54.257, 54, 1.005	3.593	6.628
Oktibbeha County, MS	1.492 (0.210)	45.122, 51, 0.885	3.242	4.637
Washington County, NC	2.683 (0.295)	17.863, 53, 0.337	3.779	2.249
Payne County, OK	1.806 (0.263)	21.140, 54, 0.391	1.338	1.356
Darlington County, SC	0.925 (0.133)	31.517, 53, 0.595	1.636	7.874
Gibson County, TN	1.824 (0.456)	23.120, 53, 0.436	1.000	1.000
Potter County, TX	1.245 (0.136)	50.155, 54, 0.929	2.805	5.937
Frio County, TX	0.557 (0.095)	23.738, 5, 4.748	3.203	127.027

bSusceptibility ratio LC of population/ LC of the most susceptible population.

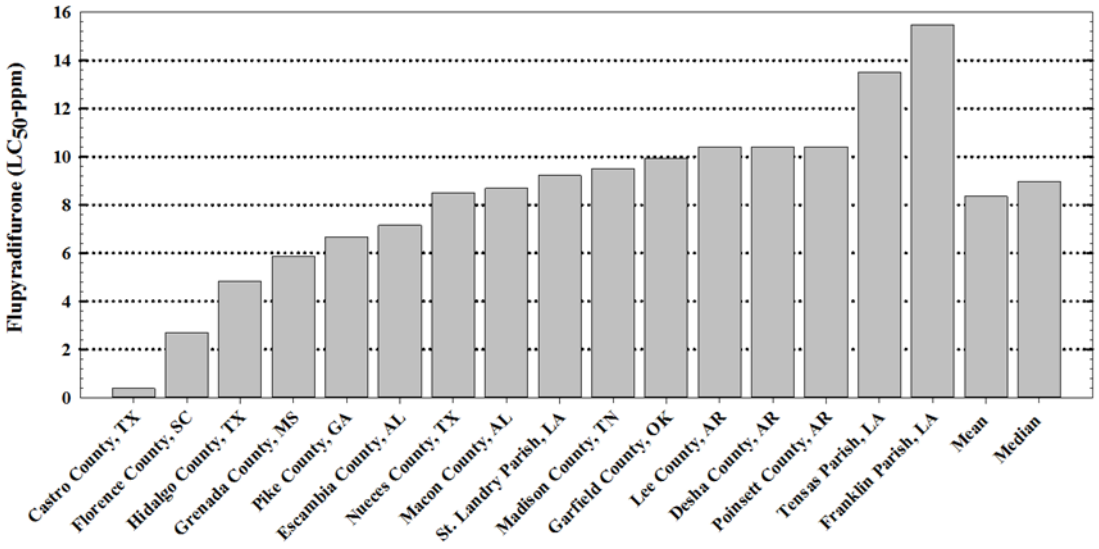

Figure V-6: 2015 Flupyradifurone LC50 values by location.

In 2016, LC_{50} values ranged from 0.32-11.11 ppm with a mean of 2.42 ppm (Figure 7). The most susceptible population was collected from Payne County, OK, and the least susceptible population was from Lawrence County, AR (Table 4). LC_{50} values in 2017 ranged from 1.50-7.85 ppm with a mean of 4.39 ppm (Figure 8). The most susceptible population was collected from Frio County, TX, while the least susceptible population was from Darlington County, SC (Table 4).

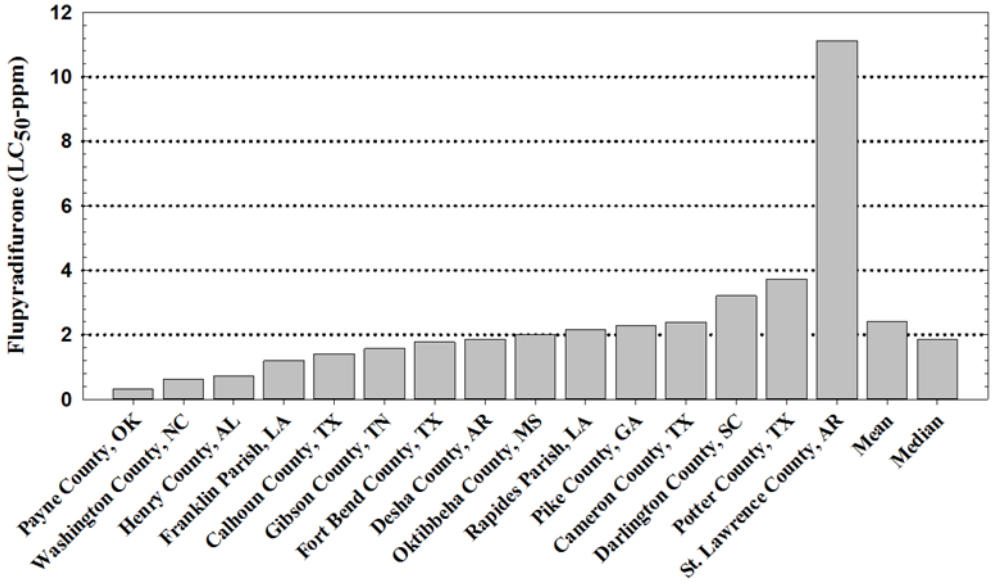

Figure V-7: 2016 Flupyradifurone LC_{50} values by location.

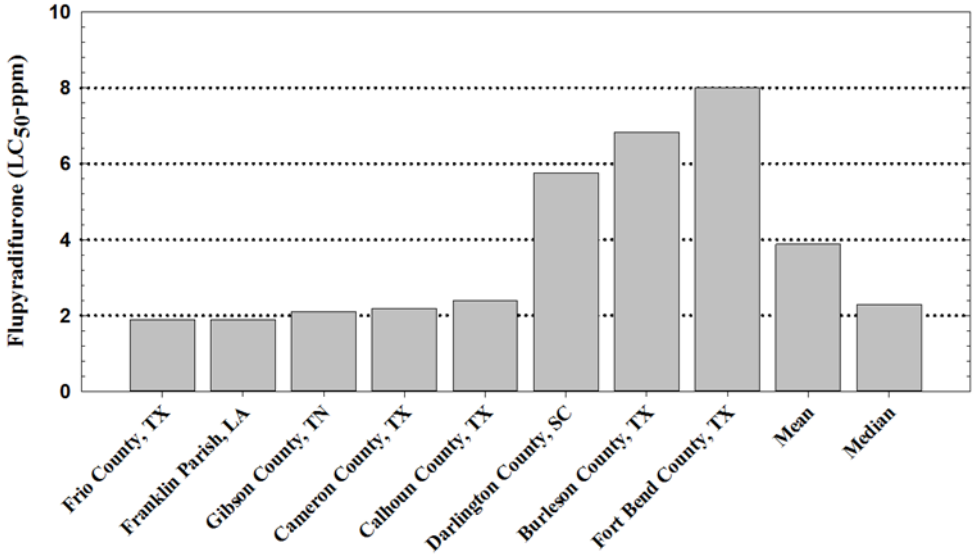

Figure V-8: 2017 Flupyradifurone LC_{50} values by location.

Table V-3: Flupyradifurone bioassay data sorted by month, year, and location for 2015.

Population	Date (M/Y)	N[a]	LC_{50}-ppm (95% Cl)[b]	LC_{90}-ppm (95% Cl)
Macon County, AL	June 2015	439	8.699 (7.130-10.254)	22.221 (19.574-26.002)
Tensas Parish, LA	June 2015	448	13.514 (10.979-16.309)	32.373 (273657-39.781)
St. Landry Parrish, LA	June 2015	445	9.229 (7.199-11.162)	23.765 (20.726-28.227)
Nueces County, TX	June 2015	487	8.507 (4.051-11.892)	24.907 (21.605-29.147)
Hidalgo County, TX	June 2015	486	4.845 (1.491-7.446)	28.220 (23.927-35.019)
Escambia County, AL	July 2015	416	7.156 (4.511-9.445)	25.173 (21.525-30.772)
Poinsett County, AR	July 2015	486	10.420 (7.290-13.685)	24.160 (19.630-32.698)
Grenada County, MS	July 2015	464	5.850 (-5.026-12.029)	27.781 (19.902-50.693)
Florence County, SC	July 2015	378	2.695 (-5.208-6.795)	16.042 (12.191-22.659)
Lee County, AR	August 2015	471	10.402 (8.926-11.955)	24.933 (22.192-28.705)
Desha County, AR	August 2015	466	10.416 (7.406-13.274)	33.308 (28.047-41.933)
Franklin Parish, LA	August 2015	449	15.491 (7.523-22.134)	44.565 (35.827-60.658)
Garfield County, OK	August 2015	426	9.939 (8.374-11.556)	22.223 (19.715-25.678)
Madison County, TN	August 2015	493	9.516 (6.645-11.924)	22.800 (20.071-26.453)
Castro County, TX	August 2015	387	0.380 (-16.672-5.94)	34.028 (23.618-76.623)
Pike County, GA	October 2015	541	6.668 (5.819-7.531)	13.557 (12.234-15.338)

[b]Susceptibility ratio LC of population/ LC of the most susceptible population.

Table V-3 Continued: Sivanto bioassay data sorted by month, year, and location for 2015.

Population	Slope (SE)	Chi-square, DF, Heterogeneity	SR-LC_{50}[b]	SR-LC_{90}[c]
Macon County, AL	0.095 (0.009)	57.756, 53, 1.089	27.528	5.626
Tensas Parish, LA	0.068 (0.006)	87.516, 52, 1.683	42.766	8.196
St. Landry Parrish, LA	0.088 (0.008)	77.626, 53, 1.465	29.206	6.016
Nueces County, TX	0.078 (0.010)	46.336, 53, 0.874	26.921	6.306
Hidalgo County, TX	0.055 (0.006)	63.819, 53, 1.204	15.332	7.144
Escambia County, AL	0.071 (0.008)	71.202, 53, 1.343	22.646	6.373
Poinsett County, AR	0.093 (0.008)	225.27, 54, 4.172	32.975	6.116
Grenada County, MS	0.058 (0.006)	409.92, 54, 7.591	18.513	7.033
Florence County, SC	0.096 (0.013)	93.785, 37, 2.535	8.528	4.061
Lee County, AR	0.088 (0.008)	35.748, 53, 0.674	32.918	6.312
Desha County, AR	0.056 (0.006)	83.574, 53, 1.577	32.962	8.432
Franklin Parish, LA	0.044 (0.007)	75.766, 53, 1.429	49.022	11.282
Garfield County, OK	0.104 (0.009)	61.419, 53, 1.159	31.453	5.626
Madison County, TN	0.096 (0.010)	56.084, 53, 1.058	30.114	5.772
Castro County, TX	0.038 (0.008)	100.29, 46, 2.180	1.203	8.615
Pike County, GA	0.375 (0.062)	48.964, 54, 0.907	21.101	3.432

[c]Susceptibility ratio LC of population/ LC of the most susceptible population.

Table V-4: Flupyradifurone bioassay data sorted by month, year, and location for 2016-2017.

Population	Date (M/Y)	N[a]	LC_{50}-ppm (95% Cl)[b]	LC_{90}-ppm (95% Cl)
Rapides Parish, LA	June 2016	466	2.171 (0.655-4.049)	214.790 (66.368-3957.800)
Fort Bend County, TX	June 2016	426	1.776 (1.284-2.309)	13.267 (10.017-18.989)
Calhoun County, TX	June 2016	501	1.404 (0.771-2.109)	16.182 (11.148-27.387)
St. Lawrence County, AR	July 2016	408	11.113 (7.857-13.979)	49.172 (34.213-101.860)
Cameron County, TX	July 2016	436	2.384 (1.809-3.027)	8.799 (6.705-12.552)
Franklin Parish, LA	July 2016	512	1.190 (0.750-1.679)	19.320 (13.478-31.526)
Henry County, AL	August 2016	456	0.725 (0.373-1.134)	10.755 (7.450-17.703)
Desha County, AR	August 2016	455	1.860 (1.233-2.554)	27.196 (18.636-45.803)
Pike County, GA	August 2016	451	2.282 (1.569-3.078)	15.809 (11.284-24.929)
Oktibbeha County, MS	August 2016	258	2.001 (1.453-2.577)	7.886 (6.059-11.146)
Washington County, NC	August 2016	440	0.618 (0.406-0.846)	3.950 (2.957-5.713)
Payne County, OK	August 2016	471	0.316 (0.077-0.684)	9.472 (5.858-18.351)
Darlington County, SC	August 2016	436	3.210 (2.190-4.245)	20.846 (15.228-32.622)
Gibson County, TN	August 2016	447	1.573 (0.884-2.228)	7.504 (5.709-10.921)
Potter County, TX	August 2016	505	3.721 (2.536-4.966)	31.406 (22.506-50.318)
Burleson County, TX	2017	532	6.830 (3.885-10.648)	31.700 (18.548-95.112)
Calhoun County, TX	2017	624	3.630 (0.139-8.473)	21.330 (9.109-831.757)
Cameron County, TX	2017	605	2.19 (1.357-3.100)	10.060 (7.175-15.725)
Darlington County, SC	2017	640	7.850 (0.005-13.265)	46.200 (25.323-66,100)
Franklin Parish, LA	2017	636	3.650 (0.322-6.381)	12.120 (7.056-58.298)
Frio County, TX	2017	650	1.500 (0.284-3.275)	28.840 (12.957-166.804)
Fort Bend County, TX	2017	596	7.760 (3.637-12.688)	64.610 (33.070-317.039)
Gibson County, TN	2017	639	1.780 (1.427-2.158)	8.410 (7.006-10.394)

[b]Susceptibility ratio LC of population/ LC of the most susceptible population.

Table V-4 Continued: Flupyradifurone bioassay data sorted by location for 2016-2017.

Population	Slope (SE)	Chi-square, DF, Heterogeneity	SR-LC_{50}[b]	SR-LC_{90}[c]
Rapides Parish, LA	0.642 (0.118)	78.156, 53, 1.475	6.870	54.377
Fort Bend County, TX	1.467 (0.137)	48.044, 53, 0.906	5.620	3.359
Calhoun County, TX	1.207 (0.158)	30.179, 53, 0.569	4.443	4.097
St. Lawrence County, AR	1.984 (0.378)	52.469, 53, 0.990	35.168	12.449
Cameron County, TX	2.259 (0.184)	88.700, 54, 1.643	7.544	2.228
Franklin Parish, LA	1.059 (0.111)	39.055, 53, 0.737	3.766	4.891
Henry County, AL	1.094 (0.137)	33.815, 54, 0.626	2.294	2.723
Desha County, AR	1.100 (0.115)	44.261, 53, 0.835	5.886	6.885
Pike County, GA	1.525 (0.131)	86.631, 54, 1.604	7.222	4.002
Oktibbeha County, MS	2.152 (0.250)	38.625, 48, 0.805	6.332	1.996
Washington County, NC	1.591 (0.177)	51.211, 53, 0.966	1.956	1.000
Payne County, OK	0.868 (0.141)	54.409, 54, 1.007	1.000	2.398
Darlington County, SC	1.577 (0.198)	32.861, 53, 0.620	10.158	5.277
Gibson County, TN	1.888 (0.297)	38.392, 53, 0.724	4.978	1.900
Potter County, TX	1.383 (0.161)	35.945, 54, 0.684	11.775	7.951
Burleson County, TX	1.923 (0.148)	23.211, 5, 4.642	21.614	8.025
Calhoun County, TX	1.667 (0.1557)	51.719, 5, 10.344	11.487	5.400
Cameron County, TX	1.9332 (0.025)	7.262, 5, 1.452	6.930	2.547
Darlington County, SC	1.664 (0.357)	13.441, 5, 2.688	24.842	11.696
Franklin Parish, LA	2.461 (0.298)	28.261, 5, 5.652	11.551	3.068
Frio County, TX	0.998 (0.110)	14.912, 5, 2.982	4.747	7.301
Fort Bend County, TX	1.393 (0.156)	13.143, 5, 2.629	24.557	16.357
Gibson County, TN	1.903 (0.163)	2.011, 5, 0.402	5.633	2.129

[c]Susceptibility ratio LC of population/ LC of the most susceptible population.

Box and whisker plots for susceptibility of SCA's to sulfoxaflor and flupyradifurone are shown in Figure 9. Dotted lines in the graph represent the mean of the data set, while bold lines represent the median. In 2014, the median of the data set was 2.00 ppm, while the mean was higher, at 3.64 ppm. For this set of data, 50% of the data points fell between 0.82 ppm and 4.93 ppm. The aphid population from Franklin Parish, LA represents an outlier in the set. In 2015, the median for the data set was 6.32 ppm, which was higher than the mean of 5.84 ppm. For this set of data, 50% of the data points fell between 1.94 ppm and 8.02 ppm, with Franklin Parish, LA once again representing an outlier in the set. In 2016, the median for the data set was 0.73 ppm, which was lower than the mean of 0.93 ppm. For this set of data, 50% of the data points fell between 0.38 ppm and 0.87 ppm, with Rapides Parish, LA representing an outlier in the set. When comparing populations over the three years, there appeared to be a slight shift in susceptibility from 2014 to 2015, with aphids being less susceptible to sulfoxaflor in 2015, and populations in 2016 appeared to be the most susceptible populations during that period.

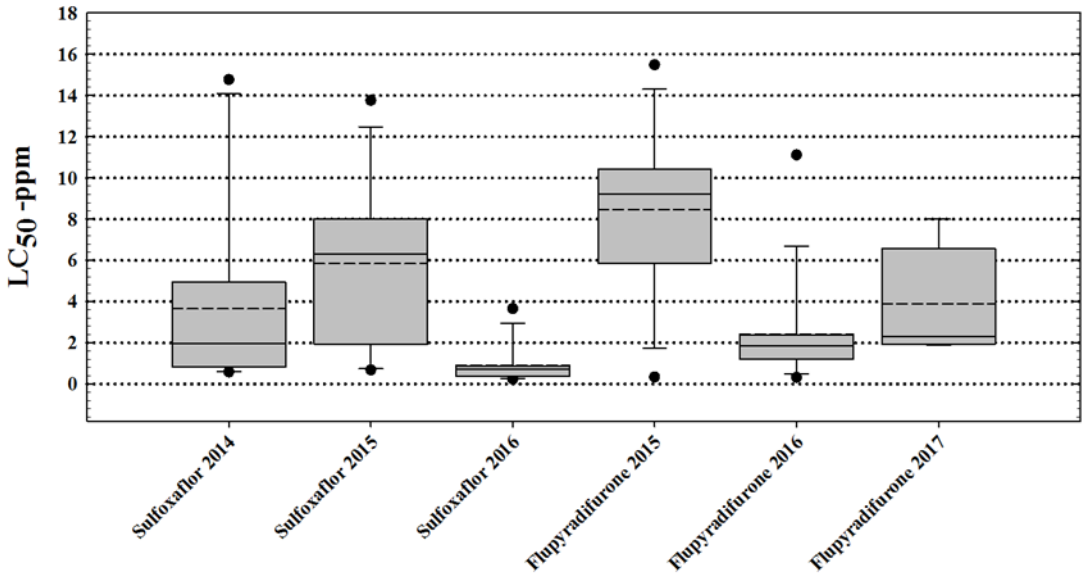

Figure V-9: Box and Whisker Plots for Susceptibility of SCA to Sulfoxaflor 2014-2016 and Flupyradifurone 2015-2017.

The data set for flupyradifurone in 2015 had a median of 9.23 ppm, higher than the mean of 8.47 ppm. For this set of data 50% of the data points fell between 5.85 ppm and 10.42 ppm. Populations from Castro County, TX and Franklin Parish, LA represent outliers in this data set. In 2016, the data set had a median of 1.86 ppm, lower than the mean of 2.57 ppm. For this set of data 50% of the populations fell between 1.19-2.38 ppm. The data set for 2017 had a median of 3.64, lower than the mean of 4.40. For this data set 50% of the populations fell between 1.89-7.53 ppm. There seems to have been a shift in the SCA's susceptibility to flupyradifurone over the three year period. In 2016 we saw a reduction in LC_{50} values overall, as populations seem to be more susceptible than populations from 2015. In 2017 we saw a slight increase in LC_{50} values, although populations do not appear to be as tolerant as 2015 populations, they do show a slightly higher tolerance compared to those from 2016. Overall, when comparing the median and

mean of flupyradifurone to those of sulfoxaflor for the 4 year period depicted by the graph, SCA's appeared to be less susceptible to flupyradifurone than sulfoxaflor. (Figure 9)

When comparing LC_{50} 's of sulfoxaflor and flupyradifurone using a correlation analysis, the relationship was significant ($P \leq 0.0001$) (Figure 10). This suggests that susceptibility to sulfoxaflor and flupyradifurone may be dependent; however, in 2016 all SCA populations appeared to be highly susceptible to both insecticides compared to years prior and 2017, which could be the reasoning for the highly significant relationship.

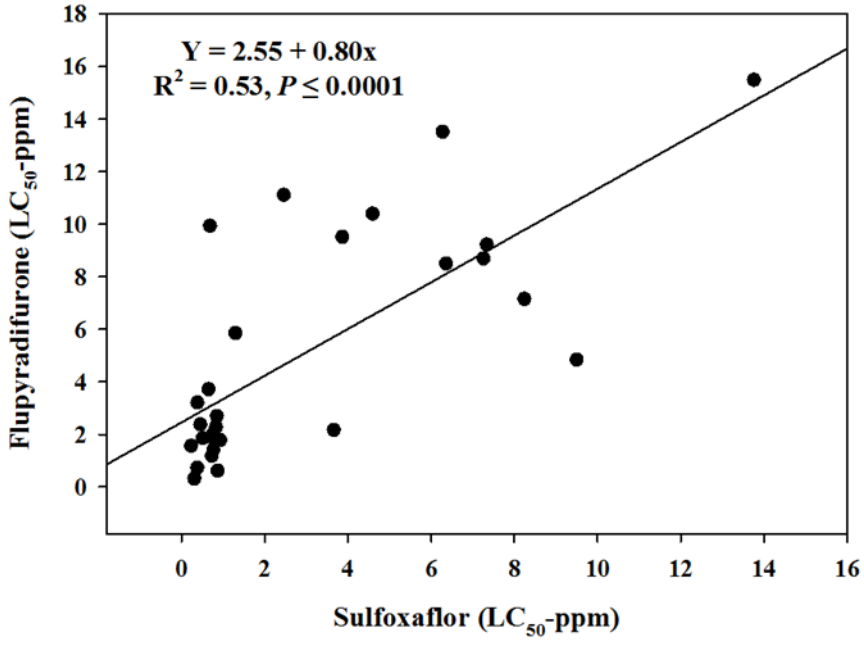

Figure V-10: Correlation Analysis for Sulfoxaflor and Flupyradifurone.

Discussion

When comparing aphid populations for susceptibility to sulfoxaflor across years, there appears to have been a slight shift in susceptibility towards higher tolerance in 2015 relative to 2014. However, in 2016 populations appeared to be the least susceptible among the three years tested.

Comparing bioassays from 2015 to 2017 for flupyradifurone, the 2016 aphid populations were more susceptible. The reason for the increase in SCA susceptibility to sulfoxaflor and flupyradifurone in 2016 is not certain; however, it may be related to a reduction in sorghum acreage, SCA infestations and/or insecticide applications targeting SCA's in 2016 relative to previous years. Overall, between 2014 and 2017 flupyradifurone appeared to be less toxic to the SCA than sulfoxaflor at equal concentrations.

In 2015 the population from Castro County, Texas and Franklin Parish, Louisiana rep- resented outliers, with Castro County representing the most susceptible population and Franklin Parish representing the least susceptible population for both insecticide data sets. The reasoning for this variability is unknown, however it could be attributed to weather and other plant and insect stress factors experienced during the growing season, but that is only speculation at this point.

In 2016, aphids appeared to be highly susceptible to both insecticides having low LC50 values throughout the season. In previous seasons it was believed that shipping and handling of aphids could lead to increased susceptibility especially in populations

traveling long distances. However, data from 2016 suggests this is not the case. In previous seasons Franklin Parish, LA populations, that did not experience shipping, appeared to be the least susceptible leading to the idea of shipping and handling having an effect on susceptibility. Data from 2016 demonstrated that, populations from Franklin Parish and Rapids Parish, LA, two locations that did not experience the shipping and handling, showed high susceptibility to both insecticides along with all other populations.

In 2017 there was 2.3 million hectares of grain sorghum planted in the United States, this is a slight decrease compared to the 2.7 million hectares planted in 2016. (NASS 2017) However the slight shift in tolerance in 2017 could be attributed to an increase in insecticide applications on these acres to control SCA populations. Multiple insects around the world have developed resistance to class 4 insecticides (Gore et al. 2013). In a similar study conducted by Gore et al. in 2013 populations of cotton aphids collected from fields previously treated with at least on foliar application experienced significantly higher LC50 values than colonies from non-treated fields. Results from the study conducted by Gore et al. in 2013 represent baseline variability of susceptibility of cotton aphid to sulfoxaflor and flonicamid. The moderate level of variability observed combined with the high level of efficacy at low rates and the high reproductive rate of the cotton aphid suggests that an effective resistance management plan needs to be devised for sulfoxaflor and flonicamid (Gore et al. 2013). Similarly, in this study, the data represents baseline susceptibility of the SCA to sulfoxaflor and flupyradifurone. The level of variability, combined with the high reproductive rate of the

SCA suggests that a similar resistance management plan needs to be implemented for these insecticides. Sulfoxaflor and flupyradifurone provide effective control of the SCA and other aphid species in areas where resistance to other group 4 insecticides occurs. However, it is important that these insecticides be incorporated into a rotation strategy to preserve their efficacy.

CHAPTER VI

RESIDUAL ACTIVITY OF CHLORPYRIFOS, SULFOXAFLOR AND FLUPYRADIFURONE ON GRAIN OSRGHUM TO THE SUGARCANE APHID

Introduction

The sugarcane aphid (SCA), Melanaphis sacchari (Zehntner), has recently become an eruptive and costly pest of sorghum in the United States. An outbreak of SCA was detected in 2013 on grain sorghum, along the Gulf Coast of Texas and Louisiana, followed closely by reports from 38 counties in four states, as well as three northeastern states of Mexico (Bowling et al. 2016; Brewer et al. 2017). The SCA rapidly displayed its ability to spread; by 2014 the aphid expanded its range to 12 states in the U.S. and over 300 counties (Bowling et al. 2016). As of fall 2015, the SCA has been confirmed on sorghum from 17 states and over 400 counties in the U.S. and all sorghum-producing regions of Mexico (Bowling et al. 2016). With the rapid spread of the SCA, control measures became an intense subject throughout the sorghum producing areas of the southern U.S. Because of the high potential for severe yield losses caused by the SCA, management with insecticides is required, as has been experienced for past aphid invasions affecting sorghum in North America (Bowling et al. 2016).

Sorghum traditionally requires relatively little water and minimal inputs, however since the SCA invasion, inputs have significantly increased due to the need for extensive control measures (Szczepaniec 2017a). Managing SCA requires the use of an

integrated approach that includes; host plant resistance, chemical control, and the use of cultural practices (Brown et al. 2015). Among cultural practices, early planting is often recommended as a means to avoid SCA infestation, or delay infestation until late in the sorghum crop's development (Singh et al. 2004; Knutson et al. 2016). Another cultural control tactic is utilizing high plant densities, which promotes low plant vigor and reduces aphid abundance (Singh et al. 2004). Landscape management is another cultural practice that can help reduce or suppress SCA populations. The host range of the SCA is largely restricted to the species of the genera: Saccharum, Sorghum, Panicum and Pennisetum (Denmark 1988). Among these Johnsongrass, Sorghum halepense, commonly grows wild in close proximity to grain sorghum fields throughout much of the sorghum producing areas of the U.S and serves as a reservoir for SCA in early spring and thus an infestation source when sorghum seedlings emerge. Elimination of nearby Johnsongrass or volunteer sorghum can be helpful in preventing SCA infestations (Knutson et al. 2016).

Over 47 species of natural enemies attack SCA worldwide, although they are often unable to prevent the development of economically damaging numbers of SCA, they are essential in slowing population development and resurgence following non-disruptive insecticide applications (van Rensburg 1979; Brewer et al. 2017). Natural enemies have demonstrated the ability to maintain SCA populations below the action threshold levels (van Rensburg 1973; Chang 1981a; Meksongsee and Chawanapong 1985; Bowling et al. 2016).

Although cultural practices and natural enemies are important in developing an integrated approach to SCA management, chemical control is often needed when the aphids reach treatable levels. Action thresholds have been developed in the southern regions of the U.S. to prevent economic impacts by SCA (Szczepaniec 2017b; Gordy et al. 2019). These action thresholds include density-based recommendations for insecticide applications at 50 SCA's per leaf, or percentages of leaves infested with SCA colonies (Brown et al. 2015; Knutson et al. 2016; Gordy et al. 2019). In addition, some action thresholds have relied on the percentage of plants infested with honeydew and SCA colonies (Catchot et al. 2015). Regardless of the action threshold utilized, it is critical that SCA not be allowed to develop high populations. Once densities exceed 500 aphids per leaf it is difficult to control SCA, and if left unmanaged they can rapidly exceed 10,000 insects per plant (Bowling et al. 2016).

Insecticides labeled for management of Hemipteran pests on sorghum prior to the SCA invasion were inconsistent in their performance (Bowling et al. 2016). Prior to 2014, chlorpyrifos (Lorsban® Advanced, Corteva Agriscience LLC, Indianapolis, IN) was one of the few commercially available insecticides registered for aphids in grain sorghum. Additional Hemipteran-specific insecticides were evaluated for efficacy to SCA during the first two years of the SCA outbreak. These evaluations led to the identification of other insecticides with satisfactory SCA efficacy. The most promising insecticides identified included sulfoxaflor (Transform® WG, Corteva Agriscience LLC, Indianapolis, IN) and flupyradifurone (Sivanto® Prime, Bayer CropScience, Research Triangle PK, NC). Sulfoxaflor received a Section 18 Emergency Exemption in most

southern states for control of the SCA on sorghum beginning in 2014. In 2015, flupyradifurone received a U.S. EPA approval for a Section 3 federal registration (Bowling et al. 2016). With only a few insecticides available to effectively manage SCA, data on efficacy and residual activity of these products is essential.

Sulfoxaflor and flupyradifurone are both IRAC class 4 insecticides, but are separated in different subgroups (IRAC 2018). Sulfoxaflor is in subgroup 4C (sulfoximines), and flupyradifurone is in subgroup 4D (butenolides) (Sparks et al. 2013; Nauen et al. 2015). Although there has not been any documented resistance of SCA to either of these insecticides, resistance management through rotation is essential in preserving the effectiveness of these products (Smith et al. 2013). Studies show there is no cross-resistance between sulfoxaflor and other insecticides, such as the neonicotinoid imidacloprid, indicating that sulfoxaflor may be a valuable tool for management of sap-feeding pests already resistant to established groups of insecticides (Longhurst et al. 2013; Wang et al. 2017). Similarly, no evidence of resistance or cross-resistance has been found for flupyradifurone, however, the long-term use of these insecticides may depend on the development of resistance management strategies that reduce the likelihood of resistance developing and increasing (Wang et al. 2018). Insecticide resistance management tactics, including chemical class rotation, use of action thresholds and other non-chemical control measures are essential to maintain the profitability and stability of agricultural production (Leach et al. 2019).

The objective of this study was to evaluate the residual activity of three insecticides utilized for managing SCA in sorghum: sulfoxaflor, flupyradifurone, and

chlorpyrifos. Results from this study can be used as a benchmark for developing properly timed insecticide rotation schemes and provide growers and pest management professionals with valuable information concerning the length of SCA control they may expect following insecticide application.

Material and Methods

In 2015 and 2016, a known susceptible grain sorghum (DGM75GB39) (Gonzales et al. 2017) was utilized to assess the field-weathered residual activity of chlorpyrifos, sulfoxalfor and flupyradifurone at the Macon Ridge Research Station in Winnsboro, LA. The 2015 trial was non-irrigated and planted on 15 May, whereas the 2016 trial was irrigated and was planted on 18 May. For both years, plots were arranged in a RCB design with 4 replicates. Plots were 4 rows × 15.2 m in length with 1.01-meter row spacing. Treatments included a non-treated control, and applications of chlorpyrifos (Lorsban® Advanced), sulfoxaflor (Transform® WG) and flupyradifurone (Sivanto® Prime) at 165.5 g [AI]/ha, 9.19 g [AI]/ha and 9.59 g [AI]/ha, respectively at approximately the pre-boot growth stage. Treatments were delivered using a 4 row, John Deere 6000 high clearance sprayer (John Deere Des Moines Works, Ankeny, IA). The sprayer were equipped with Teejet TX-6 ConeJet VisiFlo® Hollow Cone nozzles (TeeJet Technologies, Springfield, IL) (two nozzles/row), calibrated to apply 93.3 liters of finished spray per hectare. Two grain sorghum leaves were sampled from each plot. Samples were collected from the uppermost, fully expanded and exposed leaf of the

canopy, which is approximately the 9th node of the plant, at 0, 3, 7, 12, and 18 days after treatment in 2015, and at 0, 3, 7, 10, and 13 days after treatment in 2016. Leaves were sampled from the 9th node each sample date to maintain consistency.

Sampled leaves were collected into paper bags and returned to the laboratory for bioassay. The bioassay method followed a modification of that described by the IRAC susceptibility test method no. 019, (http://www.irac-online.org/content/uploads/Method_019-_v3.2_May12_aphid.pdf.) with 8 replicates per treatment, 2 leaves per plot. However, instead of the leaf dip method, we use field-weathered treated leaves to conduct the assays. Bioassay arenas consisted of individual 29-ml Solo condiment cups (Uline, Coppell, TX) with a 5-mm layer of 1.0% agar (2.0-4.5% ash) solution (Sigma-Aldrich, Co., St. Louis, MO) in the bottom. A 3-cm diameter hole was cut into each lid and sealed with a piece of single-ply tissue paper to allow excess moisture to escape. Field-weathered leaves were cut into 10-cm sections using scissors, and 3-cm leaf discs were cut from each leaf section, excluding the leaf mid-rib. Individual leaf discs were placed in bioassay cups, with the adaxial surface against the agar. A 31.75 mm outer diameter × 12.70 mm inner diameter flat steel washer was placed over the leaf disc to prevent the leaf from curling. A small paintbrush size 10/0, was used to place 10 adult SCA onto each leaf disc exposed by the inner circle of the steel washer. The SCA's utilized for the bioassay were obtained from a laboratory colony that originated from a single aphid. Each bioassay arena was held in the growth chamber (Percival Scientific, Perry IA), at 27 ± 1o C and a 12:12 L:D photoperiod.

After 48 h of exposure, mortality was scored based on the inability of aphids to show coordinated movement after being lightly prodded with a small paint brush.

Data were analyzed using PROC GLIMMIX (PROC GLIMMIX SAS Institute Inc. 2011). Data was analyzed using the random effect of insecticide*rep. A one-way analysis of variance (ANOVA) with the insecticide set as the treatment was conducted, and means were separated using Tukey's honestly significant difference (HSD) test at α=0.05. For each insecticide × year evaluation, non-linear quadratic regressions were used (SigmaPlot 12: User's Guide, 2011) to determine the relationship between corrected mortality and days of field-weathered exposure. Prior to analysis, data were corrected for mortality in the non-treated by sample date (Abbott 1925). Outliers were identified and removed using the ROUT method, with Q=1% (Motulsky and Brown 2006, GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com). LT_{50}'s (length of time for 50% mortality) was computed based on each regression model.

Results

Data from 2015 showed all three insecticides provided excellent initial mortality on the day of the application (0 days after application; 0 days after application (DAA) (Table 1). Mortality on the day of application was 96.90 ± 2.23%, 100.00 ± 0.00 % and 97.89 ± 1.41% for chlorpyrifos, sulfoxaflor and flupyradifurone, respectively. At 3 DAA, residual mortality had declined to 65.65 ± 11.32%, 62.48 ± 9.07% and 75.15 ±

4.79% for chlorpyrifos, sulfoxaflor and flupyradifurone, respectively. At this time, all insecticides resulted in higher SCA mortality than the non-treated and did not differ among each other. By 7 DAA, the mortality exhibited by chlorpyrifos and sulfoxaflor had declined to approximately 50%, and although greater than the non-treated, were significantly lower than flupyradifurone. Only flupyradifurone demonstrated significant residual activity relative to the non-treated at 12 DAA, and by 18 DAA none of the insecticides evaluated resulted in mortality different from the non-treated.

When evaluating SCA mortality over time within a single insecticide, chlorpyrifos and sulfoxaflor showed similar response. Both showed a reduction in mortality from 0 DAA to 3 DAA, but there were no differences between 3 and 7 DAA (Table 1). Differences were also observed between the 7 and 12 DAA evaluations relative to the 12 and 18 DAA evaluations, which did not differ. SCA mortality when exposed to flupyradifurone differed significantly between the day of application (0 DAA) and 3 DAA, but mortality between 3 and 7 DAA was not significant, having decreased 9%. Mortality for flupyradifurone at 12 DAA was significantly lower than at 7 DAA, and mortality at 18 DAA was significantly lower than at 12 DAA (Table 1; mean comparison within a single insecticide).

In 2016 insecticides provided statistically similar mortality on the day of application, with mortalities of 97.89 ± 1.41%, 75.64 ± 6.12% and 84.13 ± 3.29% for chlorpyrifos, sulfoxaflor and flupyradifurone, respectively (Table 1). At 3 DAA, all three insecticides had higher mortality values than the non-treated. SCA mortality on the chlorpyrifos-treated leaf tissue did not differ from the non-treated at 7 and 10 DAA,

but did differ at 13 DAA. Mortality in the sulfoxaflor treatment differed from the non-treated at 7 DAA, but not at 10 or 13 DAA. The flupyradifurone treatment resulted in significantly higher SCA mortality than the non-treated at all sample dates, and had higher mortality than chlorpyrifos and sulfoxaflor at all sample dates except at 0 DAA when it had higher mortality than sulfoxaflor, but significantly lower mortality than chlorpyrifos.

When evaluating SCA mortality over time within a single insecticide, data in 2016 showed, SCA mortality differed significantly at 3 DAA when exposed to chlorpyrifos (44.97 ± 3.77%) compared to the day of application (97.89 ± 1.41%), and also exhibited a significant reduction in mortality 7 DAA (9.78 ± 3.79%). Mortality at 10 and 13 DAA did not differ significantly, but was very low compared to the initial rating. SCA mortality, when exposed to sulfoxaflor, differed between 3 DAA and the initial evaluation at 0 DAA, and continued to decline 7 DAA but was not significantly different from 3 DAA (43.29 ± 8.35%), 10 DAA (8.00 ± 5.12%) or 13 DAA (2.00 ± 2.00%). SCA mortality, when exposed to flupyradifurone, did not differ over the first 7 days, but did experience a significant reduction in percent mortality between 7 DAA (60.50 ± 5.44%) and 10 DAA (30.89 ± 9.97%) and remained consistent out to 13 DAA (33.79 ± 3.88%) (Table 1; mean comparison within a single insecticide).

In 2015, all three insecticides exhibited a curvilinear degradation in residual activity over time (Figure 1). Using the time required to reach 50% mortality as a relative indicator, chlorpyrifos and sulfoxaflor exhibited similar LT50s of 5.93 and 5.78 days, respectively (Figure1A and B). Flupyradifurone had a longer LT50 of 10.76 days

in 2015 (Figure 1C). In 2016, residual activity of all three insecticides was shorter, but showed a similar trend as in 2015 (Figure 2). Chlorpyrifos and sulfoxaflor had LT50s of 2.69 and 2.25 days, respectively, and flupyradifurone had an LT50 of 7.65 days.

Table VI-1: Percent Mortality ± SEM of SCA when exposed to sorghum leaves treated with chlorpyrifos, sulfoxaflor or flupyradifurone at various days after application (DAA) 2015-2016.

Percent mortality ± SEM in 2015					
Treatment	0 DAA	3 DAA	7 DAA	12 DAA	18 DAA
Non-treated	5.11 ± 2.23 Ab	4.65 ± 2.54 Ab	5.25 ± 2.31 Ac	0.00 ± 0.00 Ab	1.25 ± 1.25 Aa
Chlorpyrifos 165.5 g [AI]/ha	96.90 ± 2.07 Aa	65.65 ± 11.32 Ba	47.83 ± 4.34 Bb	10.04 ± 1.92 Cb	2.36 ± 1.58 Ca
Sulfoxaflor 9.19 g [AI]/ha	100.00 ± 0.00 Aa	62.48 ± 9.07 Ba	50.29 ± 8.80 Bb	13.69 ± 4.21 Cb	5.13 ± 2.28 Ca
Flupyradifurone 9.59 g [AI]/ha	97.89 ± 1.41 Aa	75.15 ± 4.79 Ba	66.01 ± 3.88 BCa	52.52 ± 8.68 Ca	3.91 ± 2.19 Da
Percent mortality ± SEM in 2016					
Treatment	0 DAA	3 DAA	7 DAA	10 DAA	13 DAA
Non-treated	4.43 ± 2.38 Ac	2.22 ± 1.48 Ac	0.00 ± 0.00 Ac	4.54 ± 1.89 Ab	7.61 ± 2.70 Ac
Chlorpyrifos 165.5 g [AI]/ha	97.89 ± 1.41 Aa	44.97 ± 3.77 Bb	9.78 ± 3.79 Cbc	13.92 ±7.65 Cab	20.75 ± 2.79 Cb
Sulfoxaflor 9.19 g [AI]/ha	75.64 ± 6.12 Ab	43.29 ± 8.35 Bb	20.54 ± 10.36 BCb	8.00 ± 5.12 Cb	2.00 ± 2.00 Cc
Flupyradifurone 9.59 g [AI]/ha	84.13 ± 3.29 Ab	69.99 ± 3.38 ABa	60.50 ± 5.44 Ba	30.89 ± 9.97 Ca	33.79 ± 3.88 Ca

Means within a year and within columns followed by the same lower case letter and mean within a row followed by the same capital letter are not significantly different based on Tukey's HSD ($\alpha = 0.05$)

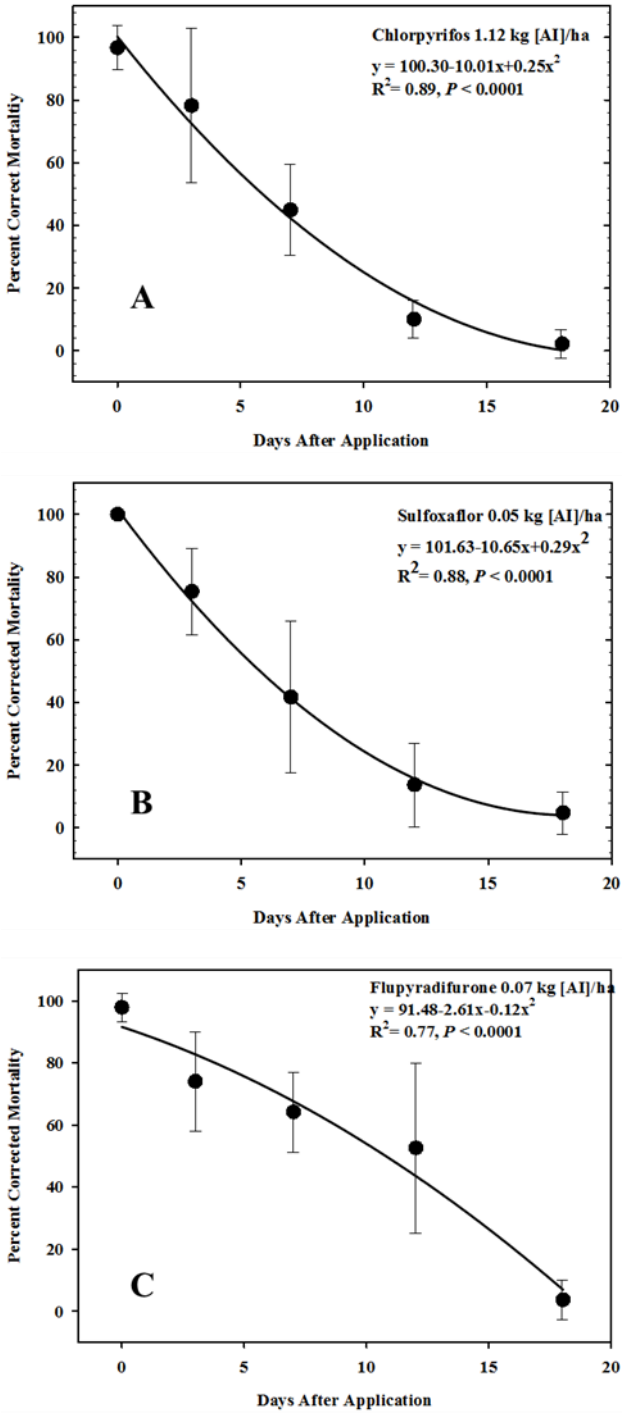

Figure 0-1: Regression Analysis, LT_{50} for Chlorpyrifos, Sulfoxaflor and Flupyradifurone 2015.

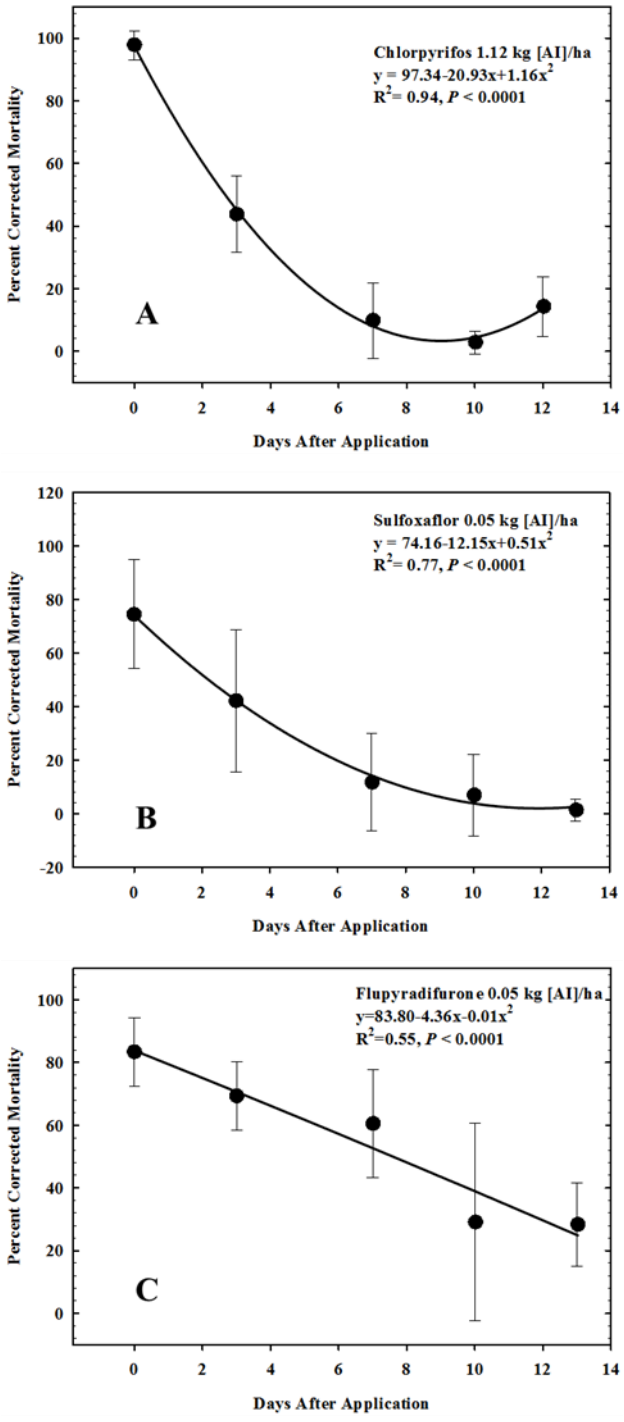

Figure 0-2: Regression Analysis, LT50 for Chlorpyrifos, Sulfoxaflor and Flupyradifurone 2016.

Discussion

The SCA is capable of explosive population growth and can cause significant damage to sorghum in a short amount of time; Currently there are only two labeled insecticides (Sivanto and Transform) for effective control of SCA as others are either marginally effective or unavailable because of pre-harvest application restrictions (Jones et al. 2016). Sorghum growers have limited insecticide options, and multiple applications of the same chemicals are being applied in the same growing season; Sivanto (flupyradifurone) is the most effective labeled product available and is safe on beneficial insects, Lorsban (chlorpyrifos) is labeled and is effective at 1 quart per acre (1.12 kg AI/ha), but producers must be aware of a harvest interval of 60 days from time of application before harvest is permitted (Smith, 2016; Jones et al., 2016). With few insecticides available for SCA control, susceptibility and residual data are important to allow future monitoring of development of resistance resulting from selection pressure from insecticide use in the field (Jones et al. 2016).

Analysis of the data from 2015 and 2016 showed high SCA mortality both years on the day of application. However, there appeared to be reduced residual activity from the insecticides in 2016 compared to 2015. It is important to note the growing and climatic conditions at the time of application during both seasons. Both years a known susceptible grain sorghum variety (Dyna-Gro M75GB39) was used to ensure antibiosis was not a factor in the mortality of the aphids. However, in 2015 the field used

experienced drought like conditions since it was non-irrigated. This may have allowed for better distribution and efficacy, considering the leaves were more erect due to the drought symptoms, taking away the difficulty of penetrating a vigorous canopy. In 2016, the hybrid was planted in an irrigated field; this posed the challenge of penetrating a vigorous canopy. However, to ensure we replicated procedures from year to year we collected from the same node (9^{th}) each sampling date. The 9^{th} node of the plant at the time of application was the uppermost, fully expanded and exposed leaf of the canopy. This should eliminate any suspicion of the insecticides failing to penetrate the canopy and sampling leaves that may not have received enough chemical to provide good toxicity to the SCA for our bioassays. In 2016, plots were sprayed following the same procedures as 2015; however after application in 2016 we received a rain only two hours after the application.

In 2015 chlorpyrifos showed to provide approximately 5 days residual activity compared to 3 days in 2016. Sulfoxaflor provided approximately 5 days in 2015 compared to 2 days in 2016. Flupyradifurone provided approximately 10 days in 2015 compared to 7 days in 2016. This data comparison would suggest that chlorpyrifos and sulfoxaflor may not provide rainfastness compared to flupyradifurone. This is an often overlooked trait in pesticides in general.

A similar study was conducted by Brittany Lipsey at Mississippi State University, where insecticides were applied in the field and SCA bioassays were conducted to evaluate the efficacy of sulfoxaflor and flupyradifurone. In that study, there was a significant relationship between percent mortality and days after treatment at

29^{o}C in both 2015 and 2016 (Lipsey, 2017). Data from this study showed in 2015 sulfoxaflor provided approximately 5 days residual activity before SCA mortality reached 50%, while flupyradifurone exceeded 10 days residual before SCA mortality reached 50% (Lipsey, 2017).

Bowling et al. in 2016 notes, these insecticides provide very high mortality of the SCA, with minimum activity of 7-10 days, and absence of economic populations of SCA up to 21 days after application. Results from these lab bioassays confirm residual activity up to 10 days for flupyradifurone and 5-7 for chlorpyrifos and sulfoxaflor, however when making application decisions it is important to evaluate the affect these insecticides have on the beneficial insect population, which provide sufficient help in controlling the SCA in the field. Chlorpyrifos, which provided great initial kill of SCA, has been proven to be very hard on beneficial insects.

In a study conducted by Smith et al. in 1985, chlorpyrifos provided greater than 98% mortality of greenbugs in sorghum, similar to SCA mortality in this study. However, results from that study conducted by Smith et al. in 1985 showed posttreatment numbers of beneficial insects were significantly higher in the check plots as a result of the absence of chlorpyrifos exposure. Both sulfoxaflor and flupyradifurone provided the same initial kill as chlorpyrifos on the day of application; however these two insecticides are more often used due to their low toxicity to aphid-specific natural enemies. Aside from adult ingestion, the impacts of sulfoxaflor and flupyradifurone on *C. carnea* were low, and although these insecticides were somewhat harmful to nymphs

and adults of *O. insidiousus*, this should not preclude their overall compatibility with biological control of the SCA (Barbosa et al, 2017).

Invasive insect pests often pose a unique challenge to crop protection, and the SCA has been especially difficult to suppress owing to their natural history traits and limited insecticide options; developing recommendations for their management that can be effectively and rapidly implemented across regions is important to minimize their impact on crops (Szczepaniec, 2018 a & b.). This study was designed to assess the residual activity of three commonly used insecticides for control of the SCA. Outcomes of this work can be used to develop an insecticide rotation strategy to develop an insecticide rotation strategy to remove selection pressure from a single insecticide. As new classes of insecticides become available for SCA control, similar research should be conducted to evaluate efficacy and residual activity to improve sustainability of sorghum production in the U.S.

CHAPTER VI

SUMMARY

The sugarcane aphid is a new pest on grain sorghum in the United States. The short generation time of aphid species often increases the likelihood of insecticide resistance due to increased exposure to these chemicals. Through this research we were able to determine multiple means of control for the sugarcane aphid on grain sorghum. By integrating many management techniques, including chemical and host plant resistance like mentioned in the above research, we are able to advise growers on the most effective control methods. Sugarcane aphid control begins in the planning process leading up to the planting of the seed, landscape management and removal of any secondary host plants, as well as other cultural practices can help reduce the potential overwintering sights of this pest. When evaluating growing conditions on the farm, selecting a hybrid that is going to offer great yield potential is first and foremost. In addition, selecting a grain sorghum hybrid that offers sugarcane aphid tolerance can help reduce the need for chemical applications during the growing season. Commercial sorghum hybrids that offer tolerance/resistance to the sugarcane aphid were found in this research, which provides growers with multiple options that may fit on their particular farm. However, through this research and other sugarcane aphid research we were able to examine and develop other cultural and chemical control strategies that can help reduce sugarcane aphid population numbers well

below economic threshold, when; scouting and decision making is done in a timely manner, therefore minimizing the need for a resistant hybrid that may limit yield potential on a particular farm. Continued monitoring and research on the sugarcane aphid is essential to ensure that this pest does not develop any resistance to the limited insecticides used for its control. Efforts by researchers and extension personnel to get information to growers about any developing issues is essential. With multiple control strategies, and a better understanding of the life cycle and habits of the sugarcane aphid we are able to stay ahead of any issues that may arise with this pest.

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