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Determining the Influence of Imidacloprid and Glufosinate Ammonium on the Population Dynamics of Twospotted Spider Mite Populations in Louisiana Cotton

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DETERMINING THE INFLUENCE OF IMIDACLOPRID AND GLUFOSINATE
AMMONIUM ON THE POPULATION DYNAMICS OF TWOSPOTTED SPIDER MITE
POPULATIONS IN LOUISIANA COTTON

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
in
The Department of Entomology

by
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B.S., Texas A&M University, 2009
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ABSTRACT

Twospotted spider mites, *Tetranychus urticae*, is an important agricultural pest of many field crops worldwide. This study investigated the impacts of imidacloprid seed treatments on populations of twospotted spider mites while also investigating if exogenous applications of jasmonic acid can offset any hormone modulating effects caused by seed treatments.

Imidacloprid seed treatments significantly increased cumulative adult mite days in 2013 but not 2015 or 2016 in the field. Applications of 10 millimolar jasmonic acid did not reduce mite severity or injury in all field trials. Imidacloprid seed treatments significantly increased all spider mite life stages in the laboratory while applications of jasmonic acid significantly reduced all mite life stages on neonicotinoid treated and non-treated cotton. Seed treatments do not affect the host preference of twospotted spider mites compared to non-treated however, jasmonic acid applications reduced the host suitability of seedling cotton to only adult mites. Additionally, leaf dip bioassays were conducted to evaluate resistance levels to abamectin in 12 populations of *T. urticae* collected from the Midsouth. Louisiana populations were highly resistant with corresponding LC₅₀ values of 0.082 and 0.184 ppm and resistance ratios of 630 and 1415-fold. One population from Mississippi was slightly resistant with an LC₅₀ value of 0.0021 ppm and a resistance ratio of 11.1 compared with a susceptible control population. Finally, greenhouse and field applied foliar spray tests and leaf dip bioassays were conducted to examine the susceptibility of *T. urticae* to glufosinate ammonium in cotton. Leaf dip bioassay results indicated that *T. urticae* were highly susceptible to concentrations of formulated glufosinate ammonium. The LC₅₀ value was determined to be 10.31 ppm. Field applied glufosinate ammonium at 1.61 and 3.14 L ha⁻¹ provided 48.86 and 80.22 percent control while fenpyroximate provided 89.62 percent control 5 days after application in 2015. Greenhouse

applications resulted in 55.43 percent control 14 days after application with 0.73 L ha⁻¹ while 1.61 L ha⁻¹ resulted in 72.86 percent control and 3.14 L ha⁻¹ resulted in 91.85 percent control of *T. urticae* populations. Data generated from these studies provide useful information on integrated pest management of twospotted spider mites in Midsouth cotton.

CHAPTER 1: INTRODUCTION AND REVIEW OF LITERATURE

The TSSM, *Tetranychus urticae* (Koch), status as an economic pest in Midsouth cotton has changed over the last 10 years. Historically, spider mites have been considered a late-season pest in the Midsouth with pesticide applications often rarely needed during early reproductive stages of cotton development (Gore et al. 2013). However, spider mites have become an increasing problem in recent years in the Midsouth (Gore et al. 2013). Numerous factors such as the use of neonicotinoid based insecticide seed treatments, use of broad-spectrum insecticides for control of other economically important pests, and inadequate or poor fall and spring vegetation management may have contributed to the increase in spider mites becoming a season-long pest in Midsouth cotton production systems.

Fungicide, insecticide and nematicide seed treatments replaced the widespread use of aldicarb (Temik 15G, Bayer Crop Science, Research Triangle Park, NC) in many fields across the Midsouth (Gore et al. 2013). The neonicotinoids thiamethoxam (Cruiser 5FS, Syngenta Crop Protection, Greensboro, NC) and imidacloprid (Gaucho Grande 5FS, Bayer Crop Science, Research Triangle Park, NC) comprise the insecticidal component of these seed treatments and have been shown to increase mite densities when compared to aldicarb or alone (Troxclair 2007, Smith et al. 2013, Szczepaniec et al. 2013). Analogous results were also documented in other crops where neonicotinoids were applied as seed treatments and foliar applications (Beers et al. 2005, Sclar et al. 1998).

Furthermore, the use of broad-spectrum insecticides for insects such as tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois) and bollworm *Helicoverpa zea* are often required to minimize economic losses. Due to widespread insecticide resistance among these pests, the practice of tank mixing organophosphates and neonicotinoids with pyrethroid insecticides is

common among many producers throughout the Midsouth. These applications disrupt beneficial arthropod populations creating an optimal environment for the proliferation of secondary pests such as TSSM (Gore et al. 2013).

Additionally, poor or inadequate fall and spring vegetation management may contribute to seasonal infestations of TSSM. TSSM have a documented host range of over 900 plant species with many of these species occurring in and around agricultural production fields in the Midsouth (Kavousi et al. 2009, Smith et al. 2013). Once these alternative hosts begin to terminate by either herbicide applications or natural senescence, spider mites will crawl to the tops of the plants to be dispersed by wind or migrate to adjacent crop hosts.

Pest Status of Twospotted Spider Mites in Louisiana

The TSSM is an important agricultural and horticultural pest of many crops worldwide (Kavousi et al. 2009, Smith et al. 2013). This arachnid has a significant host range with greater than 900 recorded species of host plants (Kavousi et al. 2009, Smith et al. 2013). In 2014, TSSM infested 83,000 acres of cotton in Louisiana resulting in applications costing \$2.34 per acre on 25,000 acres (Williams 2015). However in 2013, 128,000 acres of cotton in Louisiana were infested with TSSM resulting in applications costing \$14.96 per acre on 96,000 acres (Williams 2013). The drastic difference in application cost and TSSM incidence from 2013 to 2014 was due in part to ineffective control achieved by miticides and large amounts of precipitation received throughout the cotton production season in 2013. TSSM can also be serious pests of corn, soybeans and grain sorghum. Infestations in Louisiana's agricultural crops typically occur in fields that have late or inadequate fall vegetation management, are in close proximity to tree lines and have had prior applications of broad-spectrum insecticides for other economically

important insects. Infestations in Louisiana cotton can occur from emergence until harvest maturity.

Twospotted Spider Mite Biology

The body of the TSSM is divided into two distinct sections: the gnathosoma and idiosoma. The gnathosoma includes the mouth parts while the idiosoma contains the rest of the body and is analogous to the body of insects with the head, thorax and abdomen (Fasulo and Denmark 2009). TSSM are oval in shape, 0.50 mm in length and may possess a green – yellow hue or are almost translucent color (Fasulo and Denmark 2009). Body contents of TSSM are often visible through the transparent body wall and are composed of an accumulation of wastes that newly molted mites may lack. Females possess an elliptical body that contains 12 pairs of dorsal setae (Fasulo and Denmark 2009). While males retain an elliptical body shape, their body terminates in a caudal end that is smaller than the female (Fasulo and Denmark 2009). Eggs are small, globular objects that appear translucent and are often secured on the abaxial side of leaves with fine webbing. Larval TSSM have three pairs of legs while the following nymphal and adult stages have four. Spider mite colonies are found on the abaxial side of leaves where they are protected from rain and where temperatures are moderated. TSSM, as well as other mite species, spin fine silk webbing to attach and protect eggs and adults from predation.

The life cycle of the TSSM consists of an egg, larva, protonymph, deutonymph and adult (Cagle 1949). At the conclusion of the larval and each nymphal instar, TSSM undergo an inactive period in which the mite anchors itself to substrate and molts to the next successive stage (Shih et al. 1976). TSSM life cycle completion is highly temperature dependent requiring 7.5 days at 27 °C and 95% relative humidity (Shih et al. 1976). Mean generation time was determined to be 16.0 days with the quiescent period between deutonymph and active adult

female requiring 2.4 days (Shih et al. 1976). Reproductive rate was determined to be 7.97 eggs/female/day (Shih et al. 1976).

Shih et al. (1976) determined egg duration to be 2.3 days, larva 0.6 days, protonymph 0.4 days and deutonymph 1.9 days. Longer time spent in the egg stage results in a prolonged period for predation by phytoseiids and other egg predators, but also provides more time for older life stages to remain free of predation. Immature life stage activity resulted in larval TSSM being less active than protonymphs which exhibited less activity than deutonymphs (Shih et al. 1976). Nearing the end of the deutonymph stage, mites enter a quiescent or pharate period in which deutonymphal cuticle encloses the mite resulting in newly eclosed adult 5 – 7 days later (Shih et al. 1976). Male mites are attracted to and remain near deutonymphal females with copulation often occurring immediately after female ecdysis (Shih et al. 1976). This behavior is postulated to ensure the probability of a successful mating and increase reproductive potential under natural conditions (Shih et al. 1976). Oviposition rate reached a peak of 14.3 eggs/female/day on the 7th day and gradually decreased each day after (Shih et al. 1976). Average female oviposition rates resulted in a mean of 143.9 eggs during a 19.0 day life span (Shih et al. 1976). Therefore, females exhibited an intrinsic rate of 0.366 progeny/day (Shih et al. 1976). Conversely, Watson (1964) demonstrated longer reproductive rates and higher intrinsic rates on young plants that received a consistent nutrient supply. Intrinsic rates of increase and adult and nymphal survivorship are attributed to temperature fluctuations, relative humidity, plant age and nutrient availability of host plants. The factors outlined above coupled with the simultaneous maturation time of juveniles and rapid mating may account for the exponential growth potential of TSSM in agricultural and greenhouse settings around the world.

Only adult female TSSM have the ability to diapause (Parr and Hussey 1965).

Diapausing females are characterized by a noticeable change in color from a green – yellow during the summer months to a dark red (Parr and Hussey 1965). Three environmental factors predominately control the initiation of diapause: food availability, day length and temperature (Parr and Hussey 1965). Of the above mentioned three, day length has been shown to be the significant factor driving this process (Parr and Hussey 1965). Veerman (1977) determined that day lengths less than 14 hours resulted in significantly more TSSM entering diapause than day lengths greater than 14 hours. Under continued darkness, diapause was found to be absent (Veerman 1977). Reduced food availability and decreasing temperatures appeared to cause TSSM to enter diapause at an earlier date or in larger numbers (Parr and Hussey 1965).

Twospotted Spider Mite Injury to Cotton

TSSM are an extremely polyphagous pest that is one of the most economically important mites infesting cotton around the world. Spider mites infesting crops will usually increase in abundance, through several generations, unless kept in check by acaricides or natural enemies (Wilson 1993). TSSM feeding is characteristic of most polyphagous spider mites with injury caused by repeated piercing of plant cells with their stylets to digest cellular contents (Riley 1989). TSSM feed on the abaxial (underside) surfaces of leaves, which are major sites of photosynthesis (Welter 1989, Reddall et al. 2004). The greatest effects on yield development and yield were caused by rapidly increasing mite populations early in the growing season (Reddall et al. 2004). Wilson (1993) found a quadratic relationship existing between the rate of increase (doubling the time remaining in the production season) following infestation causes four times the amount of yield loss. These results imply that cotton is initially tolerant of mite infestations but once a critical rate of increase is exceeded, mites cause a sharp decrease in yield.

At the individual leaf level, effects of mites on photosynthesis have been investigated in a number of crops including cotton (Reddall et al. 2004). Greenhouse grown cotton plants infested with TSSM resulted in increased resistance to carbon dioxide uptake and decreased photosynthetic rate (Brito et al. 1986). Similarly, Bondada et al. (1995) evaluated field grown cotton infested with TSSM and determined internal damage to mesophyll cells and alterations to the stomatal apparatus resulted in declining photosynthesis paralleled with stomatal conductance and transpiration. Reddall et al. (2004) demonstrated reductions in stomatal conductance, rate of transpiration, photosynthetic rate and transpiration efficiency in undamaged tissue surrounding TSSM injured tissue. These findings imply that damage caused by TSSM to cotton leaves resulted in an overall decrease in photosynthesis to a greater area than just injured tissue. Furthermore, injured leaf tissue and corresponding reduced photosynthetic rate places increased competition on resources by developing bolls and squares.

Infestation timing and mite density is an important component of TSSM injury in cotton. Wilson (1993) determined the greatest decrease in flower survival occurred when severe TSSM infestations were initiated early in the fruiting period. The resulting decrease in flower survival corresponded with fewer bolls and decreased boll size (Wilson 1993). Infestations occurring later in the flower period were found to only affect boll size because setting of bolls had ceased (Wilson 1993). Effects by TSSM injury to cotton not only cause yield loss but also can have dramatic impacts on plant growth resulting in reduced germination success, crop yield, fiber quality and oil content of seeds (Wilson et al. 1991, Reddall et al. 2004). Reductions in nutritional supply, caused by TSSM injury, result in competition among seeds within a boll. This results in some seeds developing improperly, which in turn causes cotton seeds to be smaller resulting in reduced germination and oil content (Wilson 1993). Mite damage causes consistent

reductions in micronaire values, thus indicating a higher proportion of immature fibers in bolls (Rousell et al. 1951). However, fiber length is not consistently affected by TSSM injury because fiber length is determined by cell elongation, which occurs early in fiber development and may escape the effects of mites (Wilson 1993). Fiber strength was also found to not be consistently affected by mite injury (Wilson 1993). Duncombe (1977), also found no identifiable decrease in fiber strength or staple length when a 67% yield reduction was caused by mites.

Inducible Plant Immunity and the Jasmonic Acid Response

Plants are a source of nutrition for a wide array of biotic organisms in terrestrial environments. The evolution of herbivores and pathogens, with plants, has shaped the foundation of a diverse complex of specialized plant defensive compounds that exert a multitude of effects on attacking organisms (Campos et al. 2014). Inducible defensive compounds exert directly toxic, repellency or anti-nutritional effects on plant consumers, while other compounds attract natural enemies of plant associated organisms (Campos et al. 2014). A significant feature of induced defensive compounds is their ability to be expressed in tissues distal to the site of infection or attack. While constitutive defenses, such as trichome density and cuticular thickness, are confined to individual components and are expressed without activation by an external stimulus. Constitutive defenses, it is theorized, have greater resource allocation costs as compared to inducible defenses; this is due to their continuous expression throughout the plants life cycle (Thaler et al. 2012). The combined effect of the induced and constitutive defense responses provides a broad-spectrum of resistance against attack (Howe and Jander 2008, Campos et al. 2014).

Inducible defenses, to plant attack, by herbivores or pathogens are primarily composed of a suite of compounds originating from jasmonic acid (JA), salicylic acid (SA) and ethylene

pathways (ET) (Pozo et al. 2005, Lorenzo and Solano 2005, Van Loon et al. 2006, Koornneef and Pieterse 2008). Although exceptions exist, it can be generally stated that pathogens are more sensitive to SA-mediated induced defenses, whereas herbivorous insects and necrotrophic fungi are resisted more through JA/ET-mediated defenses (Thomma et al. 2001, Glazebrook 2005, Koornneef and Pieterse 2008). However, plants are often responding to multiple or simultaneous invasion by multiple aggressors which impact the primary induced defense response of the host plant (Van der Putten et al. 2001, Stout et al. 2006, Koornneef and Pieterse 2008). Therefore, plants possess regulatory mechanisms to respond and adapt to a dynamic environment. Cross talk between induced signaling pathways is theorized to provide the plant with such regulatory potential (Koornneef and Pieterse 2008). Interactions between inducible signals can be either mutually synergistic or antagonistic, resulting in positive or negative cross talk (Koornneef and Pieterse 2008). Defensive cross talk helps plants minimize resource allocation costs while also fine tuning their response to different biotic organisms (Reymond and Farmer 1998, Bostock 2005, Koornneef and Pieterse 2008).

One of the most well studied examples of defensive cross talk is the interaction between the SA and JA response pathways (Rojo et al. 2003, Bostock 2005, Koornneef and Pieterse 2008). Activation of the SA pathway should render plants more susceptible to organisms that are resisted via the JA pathway and vice versa (Koornneef and Pieterse 2008). Spoel et al. (2007) demonstrated SA mediated defenses in *Arabidopsis thaliana* were triggered upon infection of *Pseudomonas syringae*, a biotrophic pathogen that rendered infected tissues more susceptible to infection by the necrotrophic pathogen *Alternaria brassicicola* by suppressing the JA signaling pathway. Furthermore, infection by the biotrophic pathogen *Hyaloperonospora parasitica* suppressed JA mediated defenses that were initiated upon injury by *Pieris rapae* (Koornneef and

Pieterse 2008). The primary role of JA signaling for induced plant defenses can be grouped into three general observations. First, tissue injury and other forms of biotic attack result in rapid JA and jasmonoyl-L-isoleucine (JA-Ile) a JA receptor-active derivative synthesis (Campos et al. 2014). Accumulations of JA-Ile occurs in both above and below ground tissues, depending on tissue type and eliciting signal and it is also a systemic response (Chauvin et al. 2013, Fragoso et al. 2014, Campos et al. 2014). Second, the JA pathway promotes development of morphological structures in plants including resin ducts, glandular trichomes and nectaries that yield compounds responsible for direct and indirect defense roles (Van Poecke and Dicke 2002, Traw and Bergelson 2003, Hudgins et al. 2004, Leeowe et al. 2004, Campos et al. 2014). Jasmonic acid also promotes the expression of proteins and secondary metabolites involved in plant defense to abiotic stimulus including anti-nutritional proteins, terpenoids, alkaloids, phenylpropanoids, and pathogenesis proteins (Schilmiller and Howe 2005, Koo et al. 2009, Chauvin et al. 2013, Campos et al. 2014). Finally, experiments conducted with JA deficient mutants have demonstrated the pivotal role this hormone plays in plant protection against biotic organisms (Browse and Howe 2008, Campos et al. 2014).

Insecticide/Miticide Resistance in Twospotted Spider Mites

T. urticae is one of the most economically important pests in cropping systems worldwide, and is the most polyphagous species within the family Tetranychidae. With its host plants exceeding 900 plant species, insecticides and acaricides have played a primary role in controlling TSSM populations on vegetables, fruits, agricultural crops and a broad range of ornamental plants. A large number of compounds, with differing chemical structures and modes of action (MOA), have been used to control this pest; these include: neurotoxic insecticides such as organophosphates, carbamates, pyrethroids, and specific acaricides such as mitochondrial

electron transport inhibitors (METI's), avermectins and milbemycins (Van Leeuwen et al. 2009, Van Leeuwen et al. 2010). However, the TSSM is notorious for rapidly developing resistance to insecticides and acaricides (Knowles 1997, Van Leeuwen et al. 2010). Selection for resistance, in TSSM, is rapid due to its arrhenotokous reproduction, high fecundity, short life cycle and propensity for inbreeding (Van Leeuwen et al. 2009, Van Leeuwen et al. 2010). These aspects have led to the TSSM being considered the 'most resistant' in terms of total number of pesticides to which populations have become resistant according to Van Leeuwen et al. 2009.

Organophosphates were among the first chemical class used to control damaging populations of TSSM, with the first instances of control failures occurring in the 1950's (Van Leeuwen et al. 2010). Since then, TSSM has developed resistance to over 30 organophosphates and carbamates in over 40 countries (Van Leeuwen et al. 2009, Van Leeuwen et al. 2010). Organophosphate resistance in TSSM has been determined to be caused by a multitude of factors including target site insensitivity, point mutations in the AChE1 and AChE2 genes and acetylcholinesterase gene duplication (Weill et al. 2002, Oakeshott et al. 2005, Kwon et al. 2010, Van Leeuwen et al. 2010)

Pyrethroid insecticides have become one of the most widely used insecticide classes around the world accounting for 20% market share (Khambay and Jewess 2005, Van Leeuwen et al. 2010). However, resistant populations of TSSM have been reported across the world with resistance levels reaching 2000 fold over susceptible populations (Van Leeuwen et al. 2009). Several studies elucidate either enzymatic hydrolysis of carboxylesterases or oxidation by microsomal monooxygenases as the primary factors leading to the resistance of TSSM to pyrethroids (Van Leeuwen et al. 2005, Van Leeuwen and Terry 2007, Van Leeuwen et al. 2010). Furthermore, target-site resistance has also been determined to play an important role in the

formation of pyrethroid resistance as well as amino acid substitutions that lead to mutations causing destabilized pyrethroid binding to target sites (Tan et al. 2005 Tsagkarakou et al. 2009, Van Leeuwen et al. 2010).

Mitochondrial electron transport inhibitors belong to a number of chemical families including the quinazolines, pyrimidinamines, pyrazoles and pyridazinones yet share a similar MOA ; the inhibition of complex 1 of the respiratory chain (Lummen, 2007, Van Leeuwen et al. 2010). Compounds such as fenpyroximate are widely used and highly effective against all mite stages, in various crops, worldwide. METI resistance has been reported to TSSM in multiple geographic areas and crops (Van Leeuwen et al. 2009). Cross resistance to various METI's have been detected, in several instances, suggesting a common resistance mechanism (Stumpf and Nauen 2001, Van Leeuwen et al. 2010). Stumpf and Nauen (2001) theorized an oxidative mechanism that hydroxylates tertiary butyl groups attached to the heterocyclic rings, which constitute the components of all METI insecticides. Direct measurements with P450 monooxygenase activity and synergists support this theory (Stumpf and Nauen 2001, Kim et al. 2004, Van Leeuwen et al. 2010).

Abamectin Resistance in Twospotted Spider Mites

Abamectin belongs to the macrocyclic lactone family of insecticides and is produced during the fermentation of *Streptomyces avermitilis*, a soil microorganism (Burg and Stapley 1989, Riga et al. 2014). Avermectins including ivermectin and abamectin have been historically used as antiparasitic drugs for animal health applications (Riga et al. 2014). Abamectin has also been developed as a broad spectrum insecticide/acaricide with activity on Hemiptera, Diptera, Coleoptera, Lepidoptera and several mite species including *T. urticae* (Putter et al.1981). Abamectin's MOA is activation of the glutamate-gated chloride channels and is listed in Group 6

of the Insecticide Resistance Action Committee (IRAC) (Wolstenholme and Rogers 2005, Riga et al. 2014). Major crops for which abamectin is used include citrus, cotton, fruit and vegetable as well as ornamental crops.

The wide spread use of abamectin for control of TSSM has resulted in resistance development in numerous crops around the world. Resistance mechanisms in TSSM are similar to other insects which include enhanced glutathione *S*-transferase (GST), cytochrome P450-dependent monooxygenases (MFO), reduced penetration of acaricides and insecticides and target site resistance (Knowles 1997, Stumpf and Nauen 2001). Stumpf and Nauen (2001) demonstrated significantly enhanced cytochrome P450- dependent monooxygenases in resistant compared to susceptible strains of TSSM. The authors also determined that resistance, in one population, was not stable in the laboratory over six months and loss of resistance coincided with a decrease in MFO and GST activity (Stumpf and Nauen 2001). Furthermore, Stumpf and Nauen (2001) also concluded that pre-treatment with profenophos did not affect resistance to abamectin indicating that hydrolytic mechanisms may not be involved. Kwon et al. (2010) determined that a point mutation in the glutamate-gated chloride channel conferred resistance to abamectin and reciprocal crossings indicated that resistance was incompletely recessive.

Intensive applications of abamectin for control of TSSM have been used in cotton over the past decade in Louisiana. Recently, growers have observed reduced efficacy and shortened residual control indicating a possible issue with resistance development. Abamectin's fast activity and economic cost have made repeat and consecutive applications more frequent as incidence of TSSM infestations have increased in Louisiana cotton.

Glufosinate Ammonium's Role in Twospotted Spider Mite Suppression in Field Crops

Tetranychus urticae (Koch) is one of the most economically important arthropods infesting agricultural crops in the Midsouth. TSSM are often serious pests of corn, cotton, soybeans and grain sorghum. In 2015, infestations of TSSM in Midsouth cotton resulted in applications of acaricides on 420,350 acres with control costs totaling \$10.55 per acre resulting in 29,859 bales lost to this arthropod (Williams 2015). If not managed properly, TSSM injury can cause reductions in yield, lint quality, oil content in seeds and photosynthetic capacity of injured leaves (Wilson et al. 1991, Reddall et al. 2004).

Infestations in Louisiana's agricultural crops typically occur in fields that have late or inadequate fall and spring vegetation management, are in close proximity to tree lines and have had prior applications of broad-spectrum insecticides for other economically important insects. Infestations in cotton can occur from emergence until harvest maturity. Control of TSSM is primarily dependent on applications of acaricides that are often expensive and selective to only spider mites. Repeated use of the same modes of action often lead to reduced susceptibility and resistance in the target arthropod. Therefore, an integrated approach to TSSM management in field crops helps reduce dependency on acaricides, facilitates natural enemy establishment and reduces input costs to agricultural producers.

One such approach is weed management prior to planting and throughout the production season. Ahn et al. 1997 demonstrated acaricidal activity of glufosinate ammonium to populations of TSSM in apple orchards in Korea. The authors concluded that glufosinate ammonium effectively controlled all life stages of TSSM with the exception of eggs (Ahn et al. 1997). Paraquat dichloride and glyphosate were also examined for acaricidal activity however neither compound provided significant reductions in eggs, larva, protonymphs or adults (Ahn et al.

1997). Ahn et al. 1997 also reported a decrease in total acaricide applications (6 applications to 1) throughout the production season when glufosinate was substituted for conventional herbicides.

Glufosinate-tolerant or GlyTol™+ Liberty Link® (LL) cotton was commercially released in 2004 (Irby et al. 2013). Glufosinate-tolerant cotton was developed by Bayer CropScience and is resistant to post emergence applications glufosinate ammonium (Liberty® 280 SL, 24.5% [ai wt/v]; Bayer CropSciences, Research Triangle Park, NC). Glufosinate is a non-selective herbicide with activity on many grasses and broad-leaf weeds (Irby et al. 2013). Adoption of LL cotton has increased from 1.7% of U.S. cotton acres in 2009 to 5.9% of U.S. cotton acres in 2012 (USDA NASS, 2012). However, the LL cotton adoption rate has likely increased due to the identification of glyphosate resistant Palmer amaranth and other weeds in Midsouthern states (anonymous, 2015). The broad-spectrum activity as well as the ability to control glyphosate resistant weeds has made glufosinate an important component in spring vegetation management (burndown) applications prior to planting and post emergence weed control. In addition, Smith (2010) obtained 48-80% control of TSSM populations with one application of 0.58 kg ai/ha of glufosinate in Mississippi cotton.

Furthermore, the adoption of LL soybeans in Midsouth production systems is increasing for many of the same reasons previously discussed. Unlike cotton, soybeans have no reliable control measure for TSSM in the Midsouth. Many of the labelled insecticides for control of TSSM include pyrethroids and organophosphates. Field efficacy trials conducted by the LSU AgCenter located at the Red River Research Station in Bossier City, LA demonstrated unsatisfactory control of TSSM populations at 4 and 7 days after application with bifenthrin, bifenthrin+dimethoate and bifenthrin+chlorpyrifos (unpublished, 2012). Moreover, TSSM populations tripled in all insecticide treatments 7 days after application in this study

(unpublished, 2012). Results of this trial mirror problems caused by TSSM in production soybean fields in Louisiana and the Midsouth. Therefore, non-conventional acaricidal options are warranted for control of TSSM in Midsouth soybeans.

The increased adoption of LL cotton and soybeans to combat herbicide resistant weeds and the utility of glufosinate as a non-traditional acaricide may help provide agricultural producers another option for controlling weeds and TSSM with a single application.

Neonicotinoids Effect of Twospotted Spider Mite's Population Growth

Neonicotinoid insecticides represent the fastest growing class of insecticidal chemistry introduced to the global market since the advent of pyrethroids (Jeschke and Nauen 2008). In 1990, the global insecticide market was dominated by organophosphates, pyrethroids and carbamates. However, by 2008, neonicotinoids controlled 25% of the market and rose to 27% in 2010 (Simon-Delso et al.2014). The rapid adoption of neonicotinoids is due in part to their lower binding efficiencies to vertebrate target sites, selective toxicity to arthropods, persistent and systemic nature, application versatility, lower impacts on fish and aquatic invertebrates and high water solubility (Simon-Delso et al.2014).

However, neonicotinoid applications may have negative environmental consequences. One such consequence is the ability of neonicotinoid insecticides to influence severe outbreaks of spider mites in diverse plant taxa including hemlock, elm, rose, cotton and boxwood (Szczepaniec et al. 2013). Furthermore, spider mites exposed to otherwise lethal concentrations of neonicotinoids for other detrimental insects are not controlled due to a polymorphism in their nicotinic acetylcholine receptors that convey resistance to neonicotinoids (Dermauw et al. 2012).

Historically, TSSM infestations in Louisiana cotton were an infrequent occurrence that often warranted limited acaricide applications for control. However, numbers of treated acres for TSSM has gradually increased in recent years. Numerous factors may have contributed to an increase in infestations of TSSM in cotton and one such factor may be the shift away from the use of infurrow insecticides such as aldicarb to neonicotinoid seed treatments for control of below and above ground insects. Szczepaniec et al. (2013) elucidated that foliar and seed treated applications of imidacloprid, thiamethoxam and clothianidin resulted in significantly larger populations of mites throughout the duration of the study. The authors also determined that neonicotinoid applications resulted in significantly elevated TSSM population growth rates of 27% in cotton and greater than 100% in corn and tomatoes (Szczepaniec et al. 2013). Furthermore, Smith et al. (2013) demonstrated a significant increase in TSSM 16+ days after infestation where thiamethoxam, imidacloprid and AERIS (24% imidacloprid and 24% thiodicarb, Bayer CropScience) were used as seed treatments. Aldicarb and non-treated cotton seed resulted in significantly fewer mites than all neonicotinoid seed treatments (Smith et al. 2013). Their findings also coincide with previously published reports of increased numbers of spider mites in Washington apple orchards (Beers et al. 2005) and field grown marigolds (Sclar et al. 1998) following neonicotinoid applications. Moreover, Troxclair et al. (2007) observed a significantly larger percentage of cotton plants infested with TSSM in plots treated with thiamethoxam and imidacloprid seed treatments than non-treated or aldicarb treated plants.

Numerous hypotheses have been proposed to expound on the relationship between neonicotinoids and spider mite outbreaks. Pyke and Thompson (1986) demonstrated no effect of applications of neonicotinoids on *Orius insidiosus*, a generalist predator, on *Euonymus japonicas*. Furthermore, Mizell and Sconyers (1992) found that applications of imidacloprid

displayed no harmful effects to adult predatory mites *Neoseiulus collegae* (De Leon) and *Phytoseiulus macropilis* (Banks). These studies demonstrate that neonicotinoid insecticides may have little impact on populations of TSSM predators and predator removal may not be the causative agent for spider mite outbreaks. If predator removal is not a significant factor in spider mite outbreaks, another factor worthy of consideration is the effects neonicotinoids have on phytohormone expression in plants.

Inducible defenses to plant attack by herbivores or pathogens are primarily composed of a suite of compounds originating from jasmonic acid (JA), salicylic acid (SA) and ethylene pathways (ET) (Pozo et al. 2004, Lorenzo and Solano 2005, Van Loon et al. 2006, Koornneef and Pieterse 2008). Although exceptions exist, it can be generally stated that pathogens are more sensitive to SA-mediated induced defenses, whereas herbivorous insects and necrotrophic fungi are resisted more through JA/ET-mediated defenses (Thomma et al. 2001, Glazebrook 2005, Koornneef and Pieterse 2008). However, plants are often responding to multiple or simultaneous invasion by multiple aggressors which impact the primary induced defense response of the host plant (Van der Putten et al. 2001, Stout et al. 2006, Koornneef and Pieterse 2008). Therefore, plants possess regulatory mechanisms to respond and adapt to a dynamic environment. Cross talk between induced signaling pathways is theorized to provide the plant with such regulatory potential (Koornneef and Pieterse 2008). Interactions between inducible signals can be either mutually synergistic or antagonistic, resulting in positive or negative cross talk (Koornneef and Pieterse 2008). Furthermore, Ford et al. (2010) demonstrated applications of imidacloprid and clothianidin induced the salicylic acid pathway (SA) and its associated metabolites in *Arabidopsis thaliana*. Neonicotinoids, in some cases, have been reported to enhance abiotic stress tolerance and enhance plant vigor independent of their insecticidal function (Ford et al.

2010). These attributes may be associated to the endogenous biosynthesis of SA by applications of clothianidin or by the metabolism of imidacloprid into a potent analog of SA (Ford et al. 2008).

Objectives

- I. Determine the effects of foliar applied jasmonic acid and seed applied imidacloprid on phytohormone expression and TSSM populations in cotton.
- II. Determine baseline toxicity of abamectin to Louisiana and Midsouth populations of TSSM.
- III. Evaluate the efficacy and toxicity of glufosinate ammonium on populations of twospotted spider mites.
- IV. Measure reproduction and fecundity of TSSM on imidacloprid and jasmonic acid treated cotton.
- V. Determine the effects of foliar applied jasmonic acid and seed applied imidacloprid on the infestation preference of TSSM.

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CHAPTER 2: IMPACTS OF SEED APPLIED IMIDACLOPRID AND FOLIAR APPLIED JASMONIC ACID ON POPULATION GROWTH AND HOST DETERMINATION OF TWOSPOTTED SPIDER MITES IN COTTON.

Introduction

Neonicotinoid insecticides represent the fastest growing class of insecticidal chemistry introduced to the global market since the advent of pyrethroids (Jeschke and Nauen 2008). In 1990, the global insecticide market was dominated by organophosphates, pyrethroids and carbamates. However, by 2008, neonicotinoids controlled 25% of the market and increased to 27% in 2010 (Simon-Delso et al.2014). The rapid adoption of neonicotinoids is due in part to their lower binding efficiencies to vertebrate target sites, selective toxicity to arthropods, persistent and systemic nature, versatility in application methods, low toxicity to fish and aquatic invertebrates and high water solubility (Simon-Delso et al.2014).

Neonicotinoid applications may have negative environmental consequences. One such consequence is the ability of neonicotinoid insecticides to influence severe outbreaks of spider mites in diverse plant taxa including hemlock, elm, rose, cotton and boxwood (Szczepaniec et al. 2013). Furthermore, spider mites exposed to concentrations of neonicotinoids lethal to other pest insects are not controlled due to a polymorphism in their nicotinic acetylcholine receptors that convey tolerance to neonicotinoids (Dermauw et al. 2012).

Historically, twospotted spider mites (TSSM) infestations in Louisiana cotton were an infrequent occurrence that often warranted limited acaricide applications for control. However, numbers of acres treated for TSSM has gradually increased in recent years. Numerous factors may have contributed to an increase in infestations of TSSM in cotton and one such factor may be the shift away from the use of in-furrow insecticides such as aldicarb to neonicotinoid seed treatments for control of below and above ground insects. Szczepaniec et al. (2013)

demonstrated that foliar and seed treated applications of imidacloprid, thiamethoxam and clothianidin resulted in significantly larger populations of mites throughout the duration of their study. The authors also determined that neonicotinoid applications significantly elevated TSSM population growth rates by 27% in cotton and greater than 100% in corn and tomatoes (Szczepaniec et al. 2013). Smith et al. (2013) demonstrated a significant increase in TSSM populations 16+ days after infestation where thiamethoxam, imidacloprid and Aeris (24% imidacloprid and 24% thiodicarb, Bayer CropScience, Research Triangle Park, NC) were used as seed treatments. Aldicarb and non-treated cotton seed were infested by significantly fewer mites than all neonicotinoid seed treatments (Smith et al. 2013). Their findings also coincide with previously published reports of increased numbers of spider mites in Washington apple orchards (Beers et al. 2005) and field grown marigolds (Sclar et al. 1998) following neonicotinoid applications. Troxclair et al. (2007) observed a significantly larger percentage of cotton plants infested with TSSM in plots treated with thiamethoxam and imidacloprid seed treatments than non-treated or aldicarb treated plants.

Numerous hypotheses have been proposed to explain the relationship between neonicotinoids and spider mite outbreaks. Pyke and Thompson (1986) demonstrated no effect of applications of neonicotinoids on *Orius insidiosus*, a generalist predator, on *Euonymus japonicas*. Mizell and Sconyers (1992) found that applications of imidacloprid displayed no harmful effects to adult predatory mites *Neoseiulus collegae* (De Leon) and *Phytoseiulus macropilis* (Banks). With the exception of thrips, these studies demonstrate that neonicotinoid insecticides may have little impact on populations of TSSM predators and predator removal may not be the causative agent for spider mite outbreaks. Another explanation for mite outbreaks on neonicotinoid treated plants involves the effects of neonicotinoids on phytohormone expression.

Inducible defenses to plant attack by herbivores or pathogens are primarily composed of a suite of compounds originating from jasmonic acid (JA), salicylic acid (SA) and ethylene pathways (ET) (Pozo et al. 2004, Lorenzo and Solano 2005, Van Loon et al. 2006, Koornneef and Pieterse 2008). Although exceptions exist, it can be generally stated that biotrophic pathogens are more sensitive to SA-mediated induced defenses, whereas herbivorous insects and necrotrophic fungi are resisted more through JA/ET-mediated defenses (Thomma et al. 2001, Glazebrook 2005, Koornneef and Pieterse 2008). The JA or octadecanoid pathway involves JA as an intermediate signal that is triggered by tissue wounding from phytophagous arthropods and trauma that culminates in the expression of genes responsible for producing compounds such as proteinase inhibitors, terpenoids and phenolic aldehydes (Schaller and Ryan 1995). The SA pathway, often associated with pathogen infections or attack by sucking insects utilizes SA as an intermediate signal that leads to activation of genes responsible for synthesis of pathogenic proteins (Ryals et al. 1994).

However, plants often must respond to multiple or simultaneous invasion by multiple aggressors which impact the primary induced defense response of the host plant (Van der Putten et al. 2001, Stout et al. 2006, Koornneef and Pieterse 2008). Therefore, plants possess regulatory mechanisms to respond and adapt to a dynamic environment. Cross talk between induced signaling pathways is theorized to provide the plant with such regulatory potential (Koornneef and Pieterse 2008). Interactions between inducible signals can be either mutually synergistic or antagonistic, resulting in positive or negative cross talk (Koornneef and Pieterse 2008). Conversely, the addition of JA in the form of an exogenous application may offset the effects of regulatory crosstalk. Activation of host plant resistance by JA application have been documented cotton, strawberry, grapevine, tomato and lima beans (Miyazaki et al. 2014). Exogenous

applications of JA have been shown to increase expression of defense related cotton genes GhLOX1, GhAOS and GhOPR3 (Miyazaki et al. 2014). Similarly, Li et al. (2002) reported reestablishment of herbivore resistance in tomato cultivars deficient in the octadecanoid pathway (*def-1* mutants) after exogenous applications of Methyl-JA. Moore et al. 2003 documented an increase in cell-wall bound peroxidase activity in of *R. obtusifolius* two days after JA applications. Changes in cell-wall bound peroxidase levels are responsible for cell-wall rigidity, leaf expansion and induction of systemic acquired resistance to deter herbivore grazing. (Moore et al. 2003).

Furthermore, Ford et al. (2010) demonstrated applications of imidacloprid and clothianidin induced the salicylic acid pathway (SA) and its associated metabolites in *Arabidopsis thaliana*. Neonicotinoids, in some cases, have been reported to enhance abiotic stress tolerance and enhance plant vigor independent of their insecticidal function (Ford et al. 2010). These attributes may be associated to the endogenous biosynthesis of SA by applications of clothianidin or by the metabolism of imidacloprid into a potent analog of SA (Ford et al. 2010). Therefore, we proposed neonicotinoid seed treatments cause down regulation of the JA pathway while simultaneously upregulating the SA pathway. This effect would leave cotton host plants with limited capability to upregulate JA in the presence of phytophagous arthropods due to the activation of the SA pathway. We also hypothesize exogenous applications of JA can mitigate the effects of depressed JA activation and restore inducible defense capabilities in field and laboratory grown cotton. We also propose neonicotinoid seed treatments and JA applications affect host suitability, to TSSM, when given the choice of choosing hosts to colonize after a dispersal event.

MATERIALS AND METHODS

Field Study

This study was conducted at the Macon Ridge Research Station (MRRS) near Winnsboro, LA during 2013 and 2015. Excessive precipitation prevented the establishment of TSSM populations in 2014. Phytogen 499 WRF [WideStrike® (WS; Cry1Ac, Cry1F) Dow AgroScience, Research Triangle Park, NC] non-treated cotton seed was planted in commerce silt loam. The test area consisted of 24, four-row plots 15.24 meters in length on 1.01 meter centers with treatments assigned to plots in a randomized complete block design. The study consisted of four treatments: a non-treated control, 10 millimolar concentration of jasmonic acid, 28.35 gms of etoxazole (Zeal®, 72.0% [ai wt/wt]; Valent America, Walnut Creek, CA). and 0.375 mg ai/seed of imidacloprid (Gaucho 600®, 48.7% [ai wt/wt]; Bayer CropScience, Research Triangle Park) comprising a 2×3 factorial. Factor A consisted of two treatments, imidacloprid treated seed and a non-treated, and factor B consisted of the three foliar treatments outlined previously. Foliar applications were made using a 2 nozzle per row, 3 liter back pack sprayer calibrated to deliver 93.5 liters per hectare. Seed treatments were applied by hand using a small plastic bag (1.13 kg. seed/bag using a 50% slurry). Foliar applications were applied mid-bloom when mite populations were increasing in the test area.

Ten fully expanded leaves were sampled from the top 5 nodes of the plant canopy 6 and 14 days after application (DAA). Samples were placed in a paper bag and transported to the lab to be processed. Whole plants were processed using a mite brushing machine (Model 2836M, Bioquip Products, Rancho Dominguez, CA), and adults and immatures were counted using a dissecting microscope and pooled for analysis. Mite numbers were transformed into cumulative mite days (CMD) using procedures outlined in Hull and Beers (1990). Plots were harvested

when physiological maturity was reached. All plots were kept free of non-target insects throughout the duration of the study.

Phytohormone Analysis

To quantify any changes in phytohormone production elicited by TSSM and/or treatments, ten tissue samples were randomly taken from the top 33% of the plant canopy 14 DAA in each plot. Tissue samples were combined, weighed and recorded before immersion in liquid nitrogen. Tissue samples were then placed in 2ml centrifuge tubes and packed in dry ice for shipment to the Donald Danforth Plant Institute in St. Louis, MO.

Cage Study

This study was performed at MRRS in 2016 to determine if neonicotinoid seed treatments resulted in TSSM population increases and to determine if applications of JA can mitigate the effects. The test area consisted of 48 cages arranged in a randomized complete block design and maintained in an environmentally regulated cabinet operating at 28°C and 14:10 LD configuration. The study consisted of a 2×2 factorial with factor A consisting of an imidacloprid seed treatment (0.375 mg ai/seed) and factor B consisting of a foliar 10 mM JA application.

The variety used for all treatments was PhytoGen 499 WRF. Seed treatments were applied using the same method outlined for the field trial. All seeds were planted in potting soil (Miracle Gro® Marysville, OH) and watered as needed. Cages tops were constructed out of 3.78 liter clear, plastic PET containers (ULINE®, 2015) with 10.16 cm holes drilled into each side and top. Thrips netting was secured over each hole, with hot glue, to facilitate evapotranspiration by the plants and to reduce accumulation of condensation. Cage bottoms were constructed out of 1.89 liter clear, plastic PET containers. Bottoms were filled with soil to a designated mark and

with 0.5 cm diameter watering holes on each side of the cage bottom. Two seeds were planted approximately 3.81cm in depth and allowed to germinate before being thinned to one plant per cage. After plants reached sufficient height, water proof modeling clay (Prima Plastilina®, 2014) was secured around the plant stalk to prevent any mites from escaping the confinement area.

Seven days after planting (approximately two true leaves), foliar 10 mM JA applications were made using a 2 nozzle per row, 3 liter back pack sprayer calibrated to deliver 93.54 liters/hectare. To treat plants, cages were gently removed from the growth chamber and randomly arranged in two straight lines approximately 1.02 m apart to simulate plants grown in a row on 40 inch centers. Applications of JA were conducted inside of a climate controlled laboratory facility to negate any disturbance from wind and to reduce the possibility of thrips contamination to exposed cotton plants. After application, the spray was allowed to dry for 1 hour and cage tops were re-secured and placed back into the growth chamber. Seven days after application ten field collected, 1st instar TSSM were placed on the terminal leaves with a 10/0 fine camel hair paint brush.

Ten days after application, all leaf tissue was excised and examined under a dissecting microscope for presence of all mite life stages.

Host Preference

This study was performed at MRRS in 2015 to determine if neonicotinoid treated seed and JA applications alter host preference of cotton to TSSM. The test consisted of 12 arenas arranged in a randomized complete block design and maintained in an environmentally regulated cabinet at 28°C and 14:10 LD configuration. Arenas were constructed out of poster board (Peacock®, Dallas, TX) and cut into squares measuring 30.48 × 30.48 cm. Four equidistant holes, 5.08 cm in size, were drilled in 4 opposing quadrants of the arena allowing for plants to be

inserted through the holes and sealed with waterproof modeling clay to prevent any mites from escaping the confinement area. The study consisted of a 2×2 factorial with factor A consisting of an imidacloprid seed treatment and factor B consisting of a foliar JA application. The variety used for all treatments was Phytogen 499 WRF. All seeds were planted in potting soil and watered as needed. Pots used were standard 10.16 cm (width) x 11.43 cm (height) garden pots. Pots were filled with soil to a designated mark and two seeds were planted approximately 3.81cm in depth and allowed to germinate before being thinned to one plant. Seven days after thinning, one non-treated and imidacloprid seed-treated plant were randomly selected from each arena and treated with a 10mM concentration of JA. Applications were made using a 2 nozzle per row, 3 liter back pack sprayer calibrated to deliver 93.54 liters/hectare. Selected plants were removed from the growth chamber and randomly arranged in two straight lines approximately 1.02 m apart to simulate plants grown in a row on 40 inch centers. Applications of JA were conducted inside of a climate controlled laboratory facility to negate any disturbance from wind and to reduce the possibility of thrips contamination to exposed cotton plants. After application, the spray was allowed to dry for 1 hour and pots were replaced in the growth chamber. Pots were placed in standard plastic greenhouse trays (27.94cm W × 53.34cm L × 6.35 cm D) and secured with tape to prevent movement of plants or pots throughout the duration of the experiment.

Once arenas were secured, 15 TSSM infested Fordhook 242 lima bean leaves were removed and placed in the middle of the arena. Once bean leaves had completely desiccated and are free of TSSM, they were removed. Mites were allowed to naturally distribute for 7 days. At the conclusion of the 14 days, all leaf tissue was excised and examined under a dissecting microscope for presence of all mite life stages.

Statistical Analysis

All mite and phytohormone data were analyzed using PROC GLIMMIX procedure of the Statistical Analysis System (SAS® version 9.4; SAS Institute Inc., Cary NC). Mite life stages from the field trial and preference study were analyzed independently as adults and immatures and pooled for analysis as motiles. The same process was used to analyze the results of the cage study with the addition of eggs and further pooling of all life stages to determine total mites. Where significant interactions were detected between treatments the SLICEDIFF option of the LSMEANS statement was utilized to determine if a given treatment differed in number of adult, immature, egg, motile, total mites and phytohormone concentrations for each experiment. Means were separated using Tukey's honest significant difference (HSD) at the 0.05 level of significance.

RESULTS

Field Study

In 2013, 2015 and 2016 spider mite numbers built up to damaging levels while excessive precipitation prevented field studies in 2014. Mite samples were taken 7 DAA for all years tested. In 2013, adult CMDs were significantly higher in the imidacloprid treatments compared to non-treated ($F_{1,15} = 4.76$, $P=0.04$; Figure 2.1). Foliar applications of JA and etoxazole resulted in no measurable reductions in CMDs in all mite life stages ($F_{1,15} = 1.21$, $P=0.88$; $F_{1,15} = 1.87$, $P=0.19$). Phytohormone analysis indicated that JA-Ile levels were significantly lower where JA ($F_{1,15} = 16.48$, $P=0.001$) was applied to both non-treated and imidacloprid treated seeds (Figure 2.2). Levels of JA, SA, ABA and OPDA were not significantly different across treatments and treatment combinations.

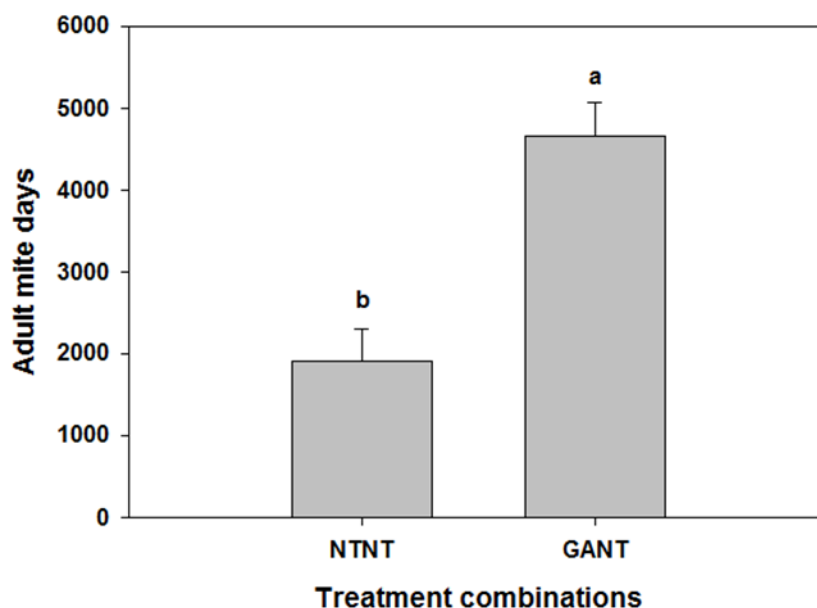


Figure 2.1 Cumulative adult mite days compared between imidacloprid/non-treated and non-treated/non-treated combinations during the 2013 field trial.

In 2015, CMDs across all treatments and treatment combinations were not statistically significant. OPDA levels were significantly higher in treatment combinations that received JA foliar applications ($F_{1,8} = 19.15$, $P=0.002$) (Figure 2.3), while all other phytohormones were not significantly different across individual treatments and treatment combinations. All plots experienced severe drought stress in 2015.

In 2016, natural infestations of TSSM failed to establish in plots resulting in artificial infestation of plots from infested soybeans. CMDs were not statistically significant for adult, immature and total motile mites across seed treatments. Foliar applications of JA resulted in no measurable differences in CMDs for adult, immature and total motiles as compared to the non-treated. Applications of etoxazole reduced CMDs in adult ($F_{1,12} = 6.67$, $P=0.02$) and total mites ($F_{1,12} = 7.70$, $P=0.01$) for the duration of this study. However, immature mites were not affected. Phytohormone levels were determined to not be significantly different across all treatments.

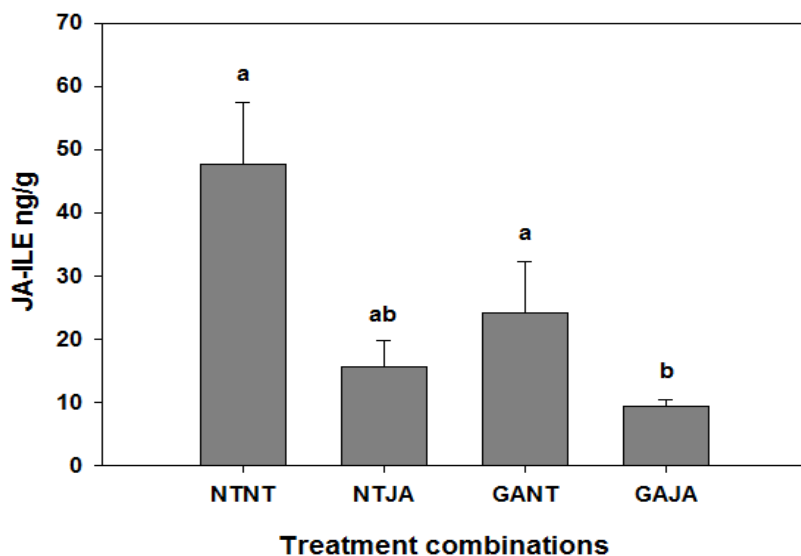


Figure 2.2. Jasmonic acid-isoleucine concentrations from neonicotinoid seed treated and non-treated cotton after applications of jasmonic acid in 2013.

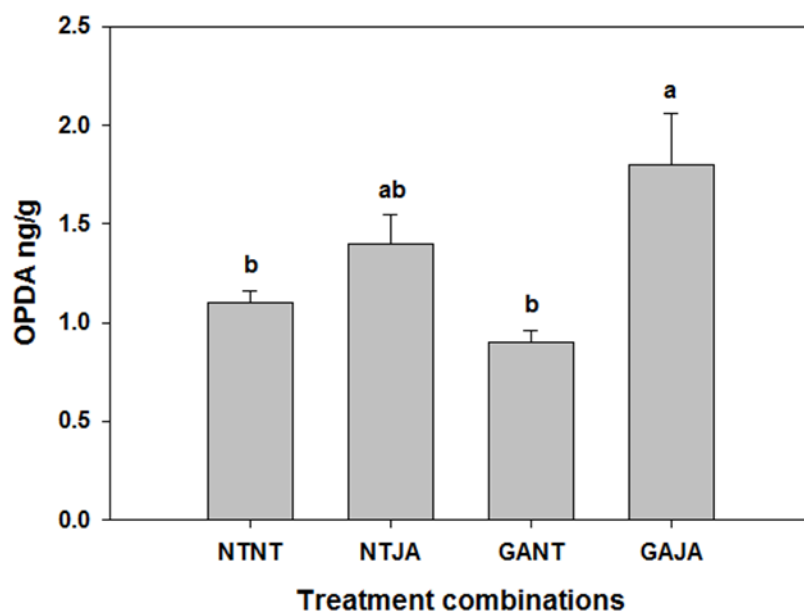


Figure 2.3. 12-oxo-phytodienoic acid concentrations from neonicotinoid seed treated and non-treated cotton applications of jasmonic acid in 2015.

Cage Study

In the cage study, the imidacloprid/non-treated combination resulted in a significant increase in all life stages of mites as compared to all other treatments combinations (Figure 2.4).

We found a significant interaction between neonicotinoid seed treatments and foliar applications of JA ($F_{1,10}= 5.39$, $P=0.04$). JA applications resulted in a significant decrease in adult, immature and egg TSSM life stages. However, adult mites in the non-treated/non-treated combination did not significantly differ than the imidacloprid/non-treated combination (Figure 2.4). Due to prohibitive cost, no phytohormone data was recorded for this experiment.

Host Preference

Imidacloprid had no significant effect on the host preference of TSSM ($F_{1,44}= 0.80$, $P=0.37$). Applications of JA reduced mite infestations significantly in adult ($F_{1,44}= 4.85$, $P=0.03$) and motile mites ($F_{1,44}= 5.15$, $P=0.03$) across all treatment combinations (Figure 2.5). JA had no significant effect ($F_{1,44}= 3.29$, $P=0.08$) on immature life stages of TSSM across treatments (Figure 2.5). Total motiles followed a similar trend to adults with reductions in total motiles attributed to JA applications. However, the reduction of overall motiles in the presence of JA is likely due to the inclusion of adults in the analysis (Figure 2.5).

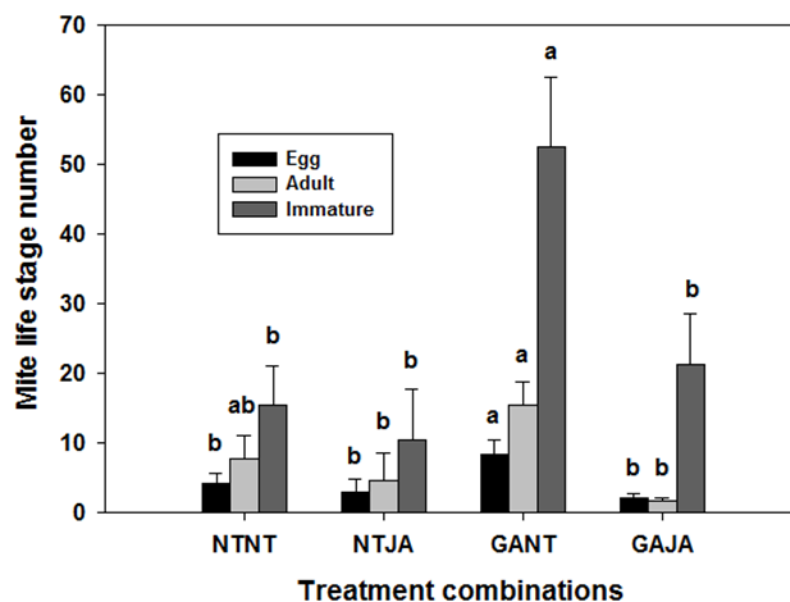


Figure 2.4. Effects of neonicotinoid seed treatment and jasmonic acid on all mite life stages.

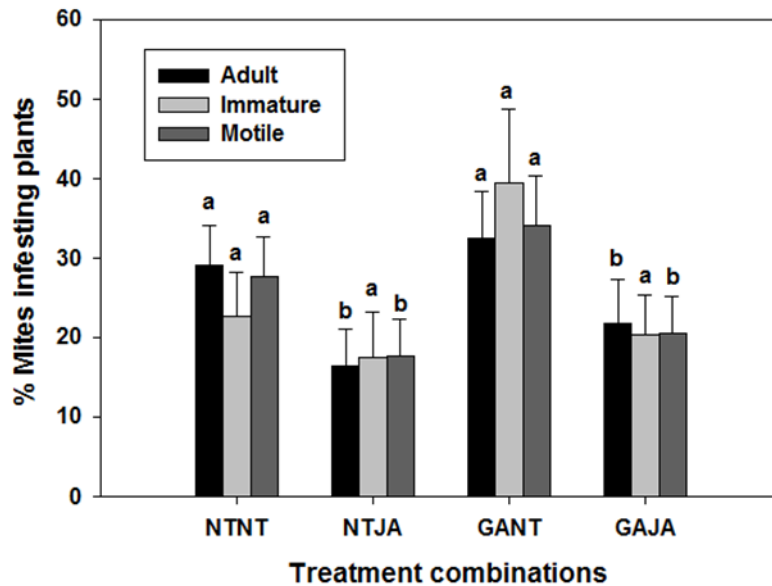


Figure 2.5. Effects of neonicotinoid seed treatment and jasmonic acid on the host preference of twospotted spider mites.

DISCUSSION

This is the first report to investigate the impacts exogenous JA applications have on neonicotinoid treated cotton seed and its effects on phytohormone expression and TSSM populations in Louisiana cotton. Applications of neonicotinoid seed treatments significantly increased the number of TSSM populations in the field and laboratory, while applications of JA caused a significant reduction in TSSM populations in the laboratory and preference studies. Our results support the hypothesis that neonicotinoid insecticides result in TSSM population increases in cotton and applications of JA can counteract the impact neonicotinoid insecticides may have on inducible plant defenses in a laboratory setting.

Imidacloprid seed treatment did not appear to significantly impact phytohormone expression in field or laboratory experiments. Levels of JA were found to be significantly higher in treatments receiving JA applications thus quantifying the absorption of JA into plant tissues after foliar applications. Phytohormone analysis results from all years tested yielded no viable

information on the synergistic or antagonistic regulation of inducible defense genes in the presence of spider mites or neonicotinoid seed treatments. This may be due to adverse environmental conditions including severe drought in 2015 and excessive precipitation in 2016. Zhu et al. (2013) observed repression of genes (DELLA protein RGA, 4-coumarate--CoA ligase like 7 and putative 12-oxophytodienoate reductase) involved in the metabolism of JA under several independent stress conditions (abscisic acid application, drought, cold, salinity and alkalinity). These results may explain the variation in phytohormone expression determined for the above mentioned field experiments.

The impacts of neonicotinoid treatments including seed, drench and foliar, on phytohormone concentrations, and inducible defenses have been previously studied in multiple crops. Szczepaniec et al. 2013 documented changes in gene expression that affect inducible defenses in cotton, corn and tomato after applications of neonicotinoids. The authors concluded that insecticide dosage may play a significant role in influencing changes in phytohormone expression with seed treatments not having a large enough amount of insecticide to alter phytohormones at a measurable level. Our study mirrors the previous author's results with elevated abundance and performance of TSSM populations across seed treatments with no measurable differences in phytohormone expression. Thus, the lack of JA suppression or SA induction is may have been caused by the small amount of insecticide used in this study.

JA is an essential compound in the octadecanoid pathway involved in induced and constitutive plant defense against herbivores. However, few studies have documented its effects on cotton as a foliar application for herbivore suppression. Zhang et al. (2011) determined mealybug females were repelled from JA treated leaves but showed no preference in host suitability on SA treated leaves. The authors also analyzed volatile emissions from JA and SA

treated plants determining an increase in methyl nicotinate and isonicotinate emissions from JA treated leaves and β -ocimene, cyclohexane, and β -caryophyllene increase from SA treated leaves. The authors concluded that gossypol production in JA treated leaves was significantly increased compared to the non-treated 5 days after treatment, while gossypol production in SA treated leaves was determined to be significantly less than the non-treated 3 days after application. Although gossypol is considered an inducible and constitutive compound regulated by the octadecanoid pathway, it demonstrated no effects on mite population growth in Agrawal and Karban (2000).

Furthermore, transcript levels of genes regulated by JA and SA were also quantified with GhLOX1 (JA dependent) and β -1,3-glucanase and acidic chitinase (SA dependent) measured after JA and SA application. The authors concluded that in response to JA application GHLOX1 transcripts were significantly induced 3 and 5 days after application; however, β -1,3-glucanase and acidic chitinase transcripts were also induced indicating that the two pathways may not be always be exclusive. These findings may indicate why TSSM were not as attracted to JA treated leaves and also explain why no statistical differences in SA and JA production were found in the phytohormone analysis from field data in this study. Additionally, Miyazaki et al. (2014) demonstrated a reduction in TSSM egg numbers (65 and 74%) after applications of JA or methyl JA to Sicot 71 (*G. hirsutum*), a TSSM susceptible cotton cultivar, in Australia. The authors also reported a 67 and 76% reduction in female mites following JA or methyl JA applications. Exogenous applications of JA or methyl JA exhibited an 80 and 85% reduction in leaf area damage as compared to control. Our cage study conclusions support the findings of Miyazaki et al. (2014) with one exception being all mite life stages were significantly reduced only in neonicotinoid treated plants and not in non-treated.

Previous studies have elucidated the effects of JA or methyl JA (JA derivative) applications on the performance of insect herbivores on treated tissue. Thaler et al. (2001) determined that foliar applications of JA resulted in early instar mortality of noctuid caterpillars and also deterred flea beetle herbivory on tomato. Similarly, Heijari et al. (2005) documented a reduction in gnawing of Scots pine by the large pine weevil after applications of 100 mM methyl jasmonate applications. However, the authors noted a reduction in height and phytotoxic effects at 100 mM methyl jasmonate rate while not at the 1 or 10 mM rate. Zhang et al. (2011) determined mealybug development time from egg to adult, on cotton treated with JA, was significantly increased compared to non-treated and SA treated leaves. Additionally, the mean weight gain of female mealybugs was significantly less on JA treated leaves compared to the control.

In conclusion, JA and its related compounds are important components in inducible defense to TSSM in cotton. Imidacloprid seed treatments caused a significant increase in all TSSM life stages in the laboratory and one-time point in the field. However, the use of an exogenous JA application can offset the effects neonicotinoid seed treatments have on population growth of TSSM in the laboratory. Applications of JA reduced the host suitability of cotton to TSSM with and without a seed treatment. Although the biochemical effects of imidacloprid seed treatments on cotton were not quantified in this study, the implications of diminished plant defenses in the presence of phytophagous arthropods is an important consideration for integrated pest management. Further research is needed into the effects insecticides have on inducible and constitutive defenses of plants.

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CHAPTER 3: SUSCEPTIBILITY OF TWOSPOTTED SPIDER MITES TO ABAMECTIN IN MIDSOUTH COTTON

INTRODUCTION

Tetranychus urticae (Koch), the twospotted spider mite (TSSM), is one of the most economically important pests in cropping systems worldwide. It is the most polyphagous species within the family Tetranychidae. With a host range exceeding 900 plant species, insecticide/acaricides have played a primary role in controlling populations of TSSM on vegetables, fruits, agricultural crops, and a broad range of ornamental plants. A variety of acaricides with differing chemical structures and modes of action have been used to control this pest. These include neurotoxic insecticides such as organophosphates, carbamates, pyrethroids, and specific acaricides such as mitochondrial electron transport inhibitors (METI's), avermectins, and milbemycins (Van Leeuwen et al. 2009, Van Leeuwen et al. 2010). However, the TSSM is notorious for rapidly developing resistance to insecticides and acaricides (Knowles 1997, Van Leeuwen et al. 2010). The risks of resistance in TSSM is enhanced by its arrhenotokous reproduction, high fecundity, short life cycle, and propensity for inbreeding (Van Leeuwen et al. 2009, Van Leeuwen et al. 2010). These aspects have led to the TSSM being considered the 'most resistant' in terms of total number of pesticides to which populations have become resistant (Van Leeuwen et al. 2009).

Abamectin belongs to the macrocyclic lactone family of insecticides/acaricides and is produced during the fermentation of *Streptomyces avermitilis*, a soil microorganism (Burg and Stapley 1989, Riga et al. 2014). Avermectins including ivermectin and abamectin have been historically used as antiparasitic drugs for applications of animal health (Riga et al. 2014). Abamectin has also been developed as a broad-spectrum insecticide/acaricide with activity on Hemiptera, Diptera, Coleoptera, Lepidoptera, and several mite species, including TSSM (Putter

et al. 1981). Abamectin acts by activating glutamate-gated chloride channels and is classified in Group 6 of the Insecticide Resistance Action Committee (IRAC) (Wolstenholme and Rogers 2005, Riga et al. 2014). Major crops for which abamectin is used include citrus, cotton, fruits and vegetables, as well as ornamental crops.

The wide spread use of abamectin for control of TSSM has resulted in resistance development in numerous crops around the world. Resistance mechanisms in TSSM are similar to other insects which include enhanced glutathione *S*-transferase (GST), cytochrome P450-dependent monooxygenases (MFO), reduced penetration of acaricides and insecticides, and target site resistance (Knowles 1997, Stumpf and Nauen 2001). Stumpf and Nauen (2001) demonstrated significantly enhanced cytochrome P450-dependent monooxygenases in resistant strains of TSSM. The authors also determined that resistance, in one population, was not stable in the laboratory over six months and loss of resistance coincided with a decrease in MFO and GST activity (Stumpf and Nauen 2001). Furthermore, Stumpf and Nauen (2001) also concluded that pre-treatment with profenophos did not affect resistance to abamectin indicating that hydrolytic mechanisms may not be involved. Kwon et al. (2010) determined that a point mutation in the glutamate-gated chloride channel conferred resistance to abamectin, and reciprocal crossings indicated that resistance was incompletely recessive.

Abamectin has been extensively used for control of TSSM in cotton over the past decade in the Midsouth. Recently, growers have observed reduced efficacy and shortened residual control, indicating a possible issue with resistance development. The fast activity and relatively low cost of abamectin products has increased their use in cotton grown in the Midsouth. Results from acaricidal efficacy trials conducted on the LSU AgCenter Macon Ridge Research Station near Winnsboro, LA (Figure 3.1), and reports of field failures with abamectin suggest that

resistance is occurring. Thus, the intent of this study was to determine the susceptibility of TSSM to abamectin for populations collected from cotton in Louisiana and the Midsouth.

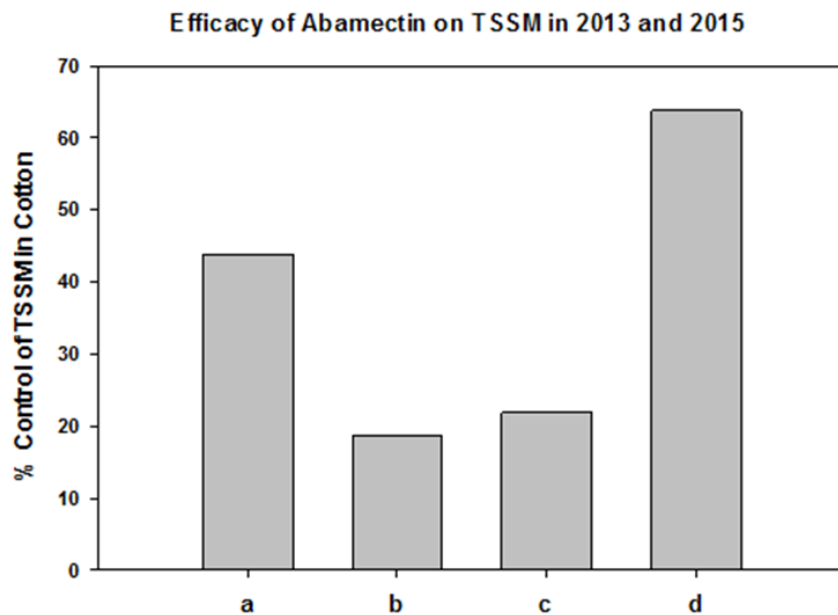


Figure 3.1 Efficacy of field applicable rates of abamectin on twospotted spider mite populations in 2013 and 2015 in Louisiana five days after application.

- a. 2013 cotton field efficacy trial previously published in Arthropod Management Tests (Brown et al. 2015).
- b. Unpublished data from field efficacy trial with abamectin (Abba® 0.15EC, 1.9% [ai wt/v]; Makhteshim Agan, Raleigh, NC) applied at 0.58 L/ha on natural populations of TSSM on cotton in 2013 near Winnsboro, LA.
- c. Unpublished data from field efficacy trial with abamectin (Abba® 0.15EC, 1.9% [ai wt/v]; Makhteshim Agan, Raleigh, NC) applied at 0.44 L/ha on natural populations of TSSM on cotton in 2015 near Winnsboro, LA.
- d. Unpublished data from field efficacy trial with abamectin (Abba® 0.15EC, 1.9% [ai wt/v]; Makhteshim Agan, Raleigh, NC) applied at 0.44 L/ha on natural populations of TSSM on cotton in 2015 near Winnsboro, LA.

MATERIALS AND METHODS

TSSM Collections

Populations of TSSM were collected from commercial cotton fields and agricultural experiment stations with suspected abamectin failures anytime during the production season

(May through September) in 2013, 2014, and 2015 at locations in Louisiana, Mississippi, Arkansas, and Tennessee (Table 3.1). Fields were independent, and no populations were taken from fields sampled in years prior. Infested leaves were excised from the upper canopy of cotton plants and placed in paper 13.34 x 8.74 x 27.79 cm paper bag (ULINE Pleasant Prairie, WI) and transported or shipped overnight to the Macon Ridge Research Station in Franklin Parish, LA. In addition to the field populations, a lab-reared strain was obtained from Dow AgroSciences, Indianapolis, IN, and used as the abamectin-susceptible control population.

Table 3.1 Description of field collected and susceptible twospotted spider mite populations by code, year, and location information. All field populations were collected in cotton.

| Population | Year | Location |
|------------|------|---|
| SUS 1 | 2013 | Dow Agrosciences, Indianapolis, IN |
| SUS 2 | 2014 | Dow Agrosciences, Indianapolis, IN |
| SUS 3 | 2014 | Dow Agrosciences, Indianapolis, IN |
| MS13 | 2013 | Production Cotton Farm, Quitman County, MS |
| TN13 | 2013 | Production Cotton Farm, Madison County, TN |
| CO13 | 2013 | Production Cotton Farm, Concordia Parish, LA |
| JV13 | 2013 | Production Cotton Farm, Catahoula Parish, LA |
| CL13 | 2014 | Production Cotton Farm, Catahoula Parish, LA |
| MS14 | 2014 | Production Cotton Farm, Washington County, MS |
| AV14 | 2014 | Production Cotton Farm, Avoyelles Parish, LA |
| MR14 | 2014 | Macon Ridge Research Station, Franklin Parish, LA |
| AR15 | 2015 | Production Cotton Farm, Drew County, AR |
| CL15 | 2015 | Production Cotton Farm, Catahoula Parish, LA |
| CL15A | 2015 | Production Cotton Farm, Catahoula Parish, LA |
| MR15 | 2015 | Macon Ridge Research Station, Franklin Parish, LA |

Infested leaves were placed in enclosed 42.4 x 27.9 x 51.2 cm cages and allowed to naturally infest *Phaseolus lunatus* (Fordhook 242 bush lima beans) potted in growing media (Miracle Gro® Marysville, OH). All cages were kept in the laboratory and placed under 121.9 x 35.4 cm grow lights (Hydrofarm® Petaluma, CA) at 26°C with 40 – 60% humidity and a

photoperiod of 16:8 (L:D). Populations of TSSM were kept free of non-target arthropods for all years tested. All populations were segregated to prevent cross contamination of individual colonies. Once sufficient numbers of TSSM were reared on lima beans, 640 adult female mites (80 per dose) were used for each bioassay.

Bioassays

Leaf-dip bioassays were conducted to evaluate the effects of abamectin (Abba® 0.15EC, 1.9% [ai wt/v]; Makhteshim Agan, Raleigh, NC) on field-collected populations of TSSM. A range of abamectin concentrations and a water control were tested on each population. Stock solutions (100 ppm active ingredient) were prepared using formulated product. Serial dilutions in water were used to obtain specific concentrations expected to kill 95% of the population at the highest concentration and 10% at the lowest level. Dose mortality for concentrations of 0.00, 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, and 0.1 parts per million were determine for each population.

New, fully expanded *P. lunatus* leaves were used for leaf-dip bioassays. All leaves were collected from plants cultivated in the greenhouse and transported to the laboratory and washed to remove any soil debris and non-target arthropods before immersion in abamectin solutions. All leaves were randomly separated by each dosage, placed on a paper towel, and allowed to air dry for 1 hour. Individual leaves were then dipped into their assigned abamectin solution for 5 seconds while simultaneously being agitated to ensure even dispersal of the acaricide. After each leaf was dipped, they were placed on paper towels, abaxial side up, and allowed to air dry for 1 hour. Once dry, individual leaves were placed abaxial side up in 100 x 15 mm Petri dishes filled with 15 mL of agar, made previously according to manufacturer's specifications (Sigma-Aldrich, St. Louis, MO), and gently pressed into the agar to ensure that the TSSM could not crawl

underneath leaves. Additionally, the agar provided moisture to the leaf, preventing desiccation. After the leaves were placed on the agar, 10 adult females of TSSM were placed on each leaf with a fine 10/0 camel hair paintbrush and each Petri dish was covered with paraffin applied to seal all gaps. The petri dishes were placed in a growth chamber operating at 27°C with 75% RH and 14:10 (L:D) photoperiod. Mortality of TSSM was assessed 48 h after infestation. Mites were examined under a dissecting microscope and considered dead when mites failed to respond to prodding with a fine 10/0 camel hair paint brush.

Statistical Analysis

Data were analyzed using a probit analysis with POLO-PLUS (LeOra 2002). Lethal concentration values were considered to be significantly different if their 95% confidence limits (CL) did not overlap. Mite mortality at each concentration was corrected based on the control mortality using the method of Abbott (1925). Resistance ratios were calculated using the formula $LC_{50} \text{ field population} \div LC_{50} \text{ susceptible population}$.

RESULTS

LC_{50} s for field collected populations of TSSM were significantly greater than susceptible populations (SUS1, SUS2, and SUS3) in each year bioassays were conducted. Mortality responses of TSSM colonies were highest for Louisiana in 2013, with LC_{50} values for CO13 and JV13 of 0.082 ppm and 0.184 ppm, respectively (Table 3.2). MS13 and TN13 were more susceptible than both JV13 and CO13 in 2013 (Table 3.2). Furthermore, MS13 and TN13 were not significantly different based on overlapping 95% confidence intervals. The calculated abamectin resistance ratios for CO13 and JV13 were very high, ranging from 631 to 1415, while the resistance ratios for TN13 and MS13 were lower ranging from 15.4 to 53.8 (Table 3.2).

Table 3.2. Mortality responses and resistance ratios of Midsouth and susceptible populations of twospotted spider mites to abamectin in 2013.

| Strain ^f | n | Gen ^a | Slope (SEM) | LC ₅₀ (95% CL) ^b | X ^{2e} | RR ^c (95% CL) ^d |
|---------------------|-----|------------------|-------------|--|-----------------|---------------------------------------|
| SUS1 | 640 | --- | 1.63 (0.14) | 0.00018 (0.00004 - 0.00024) | 84.57 | --- |
| MS13 | 640 | F1 | 2.01 (0.24) | 0.017 (0.010 – 0.028) | 74.45 | 53.8 (22.1-84.6) |
| TN13 | 640 | F1 | 2.46 (0.25) | 0.002 (0.0008 – 0.011) | 77.66 | 15.4 (2.2-31.4) |
| CO13 | 640 | F1 | 2.34 (0.23) | 0.082 (0.055 – 0.115) | 121.19 | 630.7 (598.2-705.1) |
| JV13 | 640 | F1 | 2.44 (0.26) | 0.184 (0.122 – 0.267) | 163.84 | 1415.2 (1001.5 - 1696.2) |

^aGeneration tested

^bValues expressed in ppm

^cRR (Resistance Ratio): LC₅₀ of x population/LC₅₀ SUS1

^dConfidence limits of RR calculated according to Robertson and Preisler (1992). Values for two populations were considered significantly different ($p < 0.05$) if the confidence limits on the resistance ratio did not include the value of one

^eChi-square goodness of fit tests were not significant

^fPopulations tested: SUS1 (susceptible), MS13 (Mississippi population), TN13 (Tennessee population), CO13 (Louisiana population), JV13 (Louisiana population)

All populations tested in 2014 were not different in their resistance levels based on overlapping 95% confidence intervals except for CL14 (Table 3.3). CL14 exhibited the highest LC₅₀ value of 0.017 ppm, while MS14 exhibited the lowest LC₅₀ value of 0.0021 ppm (Table 2.3). MS14, AR14, and AV14 were similarly resistant to abamectin based on overlapping 95% confidence intervals, and resistance ratios ranged from 11.1 to 94.4 (Table 3.3).

Field-collected populations of TSSM in 2015 were similar in their resistance levels to abamectin based on overlapping 95% confidence intervals (Table 3.4). CL15A exhibited the highest LC₅₀ value of 0.014 ppm, while CL15 exhibited the lowest LC₅₀ value of 0.005 ppm (Table 3.4). Resistance ratios ranged from 33.3 to 93.3 (Table 3.4).

Table 3.3 Mortality responses and resistance ratios of Louisiana, Mississippi and susceptible populations of twospotted spider mites to abamectin in 2014.

| Strain ^f | n | Gen ^a | Slope (SEM) | LC ₅₀ (95% CL) ^b | X ^{2e} | RR ^c (95% CL) ^d |
|---------------------|-----|------------------|-------------|--|-----------------|---------------------------------------|
| SUS2 | 640 | --- | 1.92 (0.17) | 0.00013 (0.00004 - 0.00024) | 61.98 | --- |
| CL14 | 640 | F2 | 2.37 (0.34) | 0.017 (0.010 – 0.028) | 97.35 | 94.4 (78.6-147.3) |
| MS14 | 640 | F1 | 3.01 (0.37) | 0.0021 (0.0010 – 0.0030) | 84.36 | 11.1 (5.2-15.1) |
| AV14 | 640 | F1 | 2.58 (0.26) | 0.0029 (0.0015 – 0.0044) | 102.32 | 16.1 (8.5-27.9) |
| MR14 | 640 | F1 | 4.13 (0.46) | 0.0024 (0.0014 – 0.0037) | 95.24 | 13.3 (2.1-31.7) |

^aGeneration tested

^bValues expressed in ppm

^cRR (Resistance Ratio): LC₅₀ of x population /LC₅₀ SUS2

^dConfidence limits of RR calculated according to Robertson and Preisler (1992). Values for two populations were considered significantly different ($p < 0.05$) if the confidence limits on the resistance ratio did not include the value of one

^eChi-square goodness of fit tests were not significant

^fPopulations tested: SUS2 (susceptible), CL14 (Louisiana population), MS14 (Mississippi population), AV14 (Louisiana population), MR14 (Louisiana population)

Table 3.4 Mortality responses and resistance ratios of Louisiana, Arkansas and susceptible populations of twospotted spider mites to abamectin in 2015.

| Strain ^f | n | Gen ^a | Slope (SEM) | LC ₅₀ (95% CL) ^b | X ^{2e} | RR ^c (95% CL) ^d |
|---------------------|-----|------------------|-------------|--|-----------------|---------------------------------------|
| SUS3 | 640 | --- | 1.89 (0.24) | 0.00015 (0.00004- 0.00024) | 69.18 | --- |
| CL15 | 640 | F1 | 2.78 (0.35) | 0.005 (0.002 – 0.011) | 103.25 | 33.3 (11.2-54.2) |
| CL15A | 640 | F1 | 3.26 (0.58) | 0.014 (0.010 – 0.020) | 97.53 | 93.3 (74.5-128.3) |
| MR15 | 640 | F1 | 2.87 (0.47) | 0.008 (0.005 – 0.011) | 85.36 | 53.3 (21.3-84.2) |
| AR15 | 640 | F2 | 2.97 (0.26) | 0.009 (0.004 – 0.013) | 88.65 | 60.0 (32.7-86.9) |

^aGeneration tested

^bValues expressed in ppm

^cRR (Resistance Ratio): LC₅₀ of x population /LC₅₀ SUS3

^dConfidence limits of RR calculated according to Robertson and Preisler (1992). Values for two populations were considered significantly different ($p < 0.05$) if the confidence limits on the resistance ratio did not include the value of one

^eChi-square goodness of fit tests were not significant

^fPopulations tested: SUS3 (susceptible), CL15 (Louisiana population), CL15A (Louisiana population), MR15 (Louisiana population), AR15 (Arkansas population)

DISCUSSION

Susceptibility of TSSM to abamectin varied significantly in populations collected from the Midsouth. These observations confirm resistance and reports of control failures experienced in Midsouth cotton fields, with populations from Louisiana exhibiting the highest LC_{50} values of all colonies tested. Populations from Concordia (CO13) and Catahoula (JV13) Parishes were collected two to four days after a second consecutive application of abamectin was made to control TSSM. CO13 had one application at 0.29 L/ha and a second 5 days later at 0.44 L/ha, while JV13 had the first application at 0.44 L/ha and a second 7 days later at 0.58 L/ha. Populations from other states and areas around Louisiana were collected after suspected field failures with abamectin; however, abamectin use rates and application timing intervals were not certain at these locations. The LC_{50} values for all years were significantly greater than those of the control populations. These values represent a general reduction in susceptibility by TSSM to abamectin regardless of year or location. Abamectin resistance mechanisms were not studied in the present research.

Abamectin resistance in TSSM has been shown to be highly variable depending on exposure and intensity of selection pressure. Ferreira et al. 2015 estimated resistance ratios ranging from 8.0 to 295,270 in prior populations collected from cotton and ornamental flower plantations in Brazil. The authors noted that no resistance management practices had been adopted, leading to the extreme levels of abamectin resistance on flower plantations (Ferreira et al. 2015). Furthermore, Sato et al. (2005) reported a resistance ratio of 25 for a population of TSSM collected from a strawberry farm in Brazil compared with a susceptible population. They noted prolific use of abamectin for the previous 10 years and documented at least 6 applications

the year the population was collected. The results obtained in this study mirror the results of the previous authors documenting selection driven resistance to acaricides.

Known resistance mechanisms in TSSM are similar to other arthropods which include enhanced glutathione *S*-transferase (GST), cytochrome P450- dependent monooxygenases (MFT), reduced penetration of acaricides and insecticides, and target site resistance (Knowles 1997, Stumpf and Nauen 2001). Moreover, results from Sato et al. 2005 and Stumpf and Nauen 2001 reported that abamectin resistance is unstable in the absence of selection pressure, although both authors demonstrated stable abamectin resistance in colonies confined to the laboratory. The instability of abamectin resistance is considered favorable for the management of resistant populations (Dennehy et al. 1990). The instability of abamectin resistance may explain the large shifts in susceptibility from year to year in this study. Therefore, enough time may elapse from one cotton season to another that some level of abamectin susceptibility is reestablished. Immigration of TSSM from other host plants may also increase susceptibility. These factors may explain the large LC_{50} values obtained from CO13 and JV13 and the significantly lower LC_{50} values observed in the populations tested for 2014 and 2015. However, populations of TSSM appear to be retaining varying levels of resistance to abamectin. Stumpf and Nauen (2001) demonstrated no significant loss of resistance to abamectin after 6 months without selection in a population collected from the Netherlands on roses. These reports indicate that the instability of resistance can't be used to generalize the response of TSSM populations in Midsouth cotton.

Aside from selection by acaricides, host crop and previously used insecticides may play a role in the influence of abamectin resistance in TSSM. Although all populations were collected in cotton, alternative host origination may impact the susceptibility of TSSM to abamectin and other insecticides used cotton production. Dermauw et al. (2012) described a shift in TSSM

transcription profiles responsible for detoxification of inducible and constitutive plant defenses and insecticides when TSSM were placed on a new host (tomato). Their findings also concluded that expression changes were much more pronounced after 5 generations than with short-term responses (hours). Thus, the origin of a TSSM population and generation time in the field may have a significant effect on susceptibility to abamectin.

Similarly, Yang et al. (2001) observed that TSSM exposed to dimethoate developed 15.9-fold resistance levels to bifenthrin when compared to non-selected mites. Insect control in Midsouth cotton employs the use of several modes of action that are often tank mixed and applied in short application windows for economically important pests such as tarnished plant bug, *Lygus lineolaris*, (Palisot de Beauvois) and bollworm, *Helicoverpa zea* (Boddie). Many of these insecticides “flare” secondary pests such as TSSM and cotton aphid, *Aphis gossypii* (Glover), by effectively removing natural enemies from the agro-ecosystem. Therefore, the combination of natural enemy removal and induced resistance from the application of insecticides targeting other pests may increase the frequency of control failures with acaricides.

Finally, the fluctuation in susceptibility may be due in part to a reduction in abamectin use as Extension efforts have focused on integrated pest management programs and rotation of acaricides for resistance management. Recommendations, based on reports of field failures and reduced efficacy with abamectin in test plots, encouraged producers and other agricultural professionals to utilize alternative chemistries for control of TSSM while also not relying on a single mode of action.

In conclusion, very high resistance levels of TSSM to abamectin were observed in some populations collected from the Midsouth states. Further studies on the stability and resistance mechanisms are warranted to elucidate the causes of abamectin resistance in populations of

TSSM. An improved understanding of abamectin resistance in TSSM is important to maintain the useful life of this chemical for the control of this pest in in the Midsouth.

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CHAPTER 4: GLUFOSINATE AMMONIUM'S ROLE IN TWOSPOTTED SPIDER MITE SUPPRESSION IN COTTON

INTRODUCTION

Tetranychus urticae (Koch) is one of the most economically important arthropods infesting agricultural crops in the Midsouth (Smith et al. 2010). Twospotted spider mites are often serious pests of corn, cotton, soybeans and grain sorghum. In 2015, infestations of TSSM in Midsouth cotton resulted in applications of acaricides on 420,350 acres with control costs totaling \$10.55 per acre and resulted in 29,859 bales lost (Williams 2015). If not managed properly, TSSM injury can cause reductions in yield, lint quality, oil content in seeds and photosynthetic capacity of injured leaves (Wilson et al. 1991, Reddall et al. 2004).

Infestations in Louisiana's agricultural crops typically occur in fields that have late or inadequate fall and spring vegetation management, are in close proximity to tree lines or have had prior applications of broad-spectrum insecticides targeting other economically important insects. Infestations in cotton can occur from emergence until harvest maturity (Gore et al. 2013). Control of TSSM is primarily dependent on applications of acaricides that are often expensive and selective to only spider mites. Repeated use of the same modes of action often lead to reduced susceptibility and resistance in the target arthropod. Therefore, an integrated approach to TSSM management in field crops helps reduce dependency on acaricides, facilitates natural enemy establishment and reduces input costs to agricultural producers.

One such approach is weed management prior to planting and throughout the production season. Gotoh (1997) demonstrated a reduction in TSSM infestations when herbicide applications were made during winter months to eliminate weeds in pear orchards. The author also concluded that winter weed management reduced overall TSSM populations infesting pear trees even in the presence of large mite populations overwintering in the bark from the previous

year (Gotoh 1997). An added benefit in TSSM control, originating from weed management, may occur where the herbicide utilized also exhibits toxicity to the spider mite. Ahn et al. 1997 demonstrated acaricidal activity of the herbicide glufosinate ammonium to populations of TSSM in apple orchards in Korea. The authors concluded that glufosinate ammonium effectively controlled all life stages of TSSM with the exception of eggs. Paraquat dichloride and glyphosate were also examined for acaricidal activity however neither compound provided significant reductions in eggs, larva, protonymphs or adult TSSM (Ahn et al. 1997). They also reported a decrease in total acaricide applications (6 applications to 1) throughout the production season when glufosinate was substituted for other herbicides.

Glufosinate-tolerant or GlyTol™+ Liberty Link® (LL) cotton was commercially released in 2004 (Irby et al. 2013). Glufosinate-tolerant cotton was developed by Bayer CropScience and is resistant to post emergence applications glufosinate ammonium (Liberty® 280 SL, 24.5% [ai wt/v]; Bayer CropSciences, Research Triangle Park, NC). Glufosinate is a non-selective herbicide with activity on several grasses and broad-leaf weeds (Irby et al. 2013). Adoption of LL cotton has increased from 1.7% of U.S. cotton acres in 2009 to 5.9% of U.S. cotton acres in 2012 (USDA NASS, 2012). However, the LL cotton adoption rate has likely increased due to the identification of glyphosate resistant Palmer amaranth, *Amaranthus palmeri*, and other weeds in Midsouthern states (D. Miller personal communication). The broad-spectrum activity, as well as the ability to control glyphosate resistant weeds, has made glufosinate an important component in spring vegetation management (burndown) applications prior to planting and post emergence weed control. In addition, Smith et al. 2010 obtained 48-80% control of TSSM populations with one application of 0.58 kg-ai/ha of glufosinate in Mississippi cotton.

The increased adoption of LL cotton to combat herbicide resistant weeds and the utility of glufosinate as a non-traditional acaricide may help provide agricultural producers another option for controlling weeds and TSSM with a single application. The objectives of this study were to quantify the toxicity of glufosinate ammonium towards TSSM populations, and to determine which use rates of glufosinate ammonium exhibit activity towards TSSM in cotton, and how much control to expect from these rates relative to a commonly used acaricide.

MATERIALS AND METHODS

Foliar Efficacy

All studies were performed at the Macon Ridge Research Station (MRRS, LSU AgCenter) near Winnsboro, LA (Franklin Parish) during the 2015 and 2016 growing season. The cotton variety used for both years was Stoneville 5289 GLT (Glytol Liberty Link) and was planted on 29 May in 2015 and 15 September in 2016. For the 2015 study, all plots consisted of four rows (centered on 1.02 m) by 13.71 m in length. Treatments were arranged in a randomized complete block design with four replications. The 2016 study was conducted in a greenhouse due to TSSM populations failing to colonize cotton plants during the production season. For the greenhouse study, four Stoneville 5289 GLT cotton seeds were planted in 80 nursery pots (0.32 m x 0.28 m) filled with growing media (Miracle Gro® Marysville, OH). After emergence, plants were thinned to two per pot and watered as needed. All plants were kept between 26 and 30°C and a photoperiod of 16:8 (L:D). Once plants had reached 8 true leaves, TSSM infested *Phaseolus lunatus* (Fordhook 242 bush lima beans) were placed in pots and allowed to naturally infest the cotton plants. All plants tested were kept free of non-target arthropods for the duration of the study. Pots were placed on a level surface and oriented to simulate two rows centered on

1.02 m. Thus, each plot consisted of 4 pots totaling 8 plants arranged in a randomized complete block design and replicated 4 times.

The 2015 and 2016 cotton study consisted of three foliar glufosinate treatments, a standard acaricide control and a control treatment. Products used were glufosinate ammonium and fenpyroximate (Portal XLO®, 5.0% [ai wt/wt]; Nichino America, Wilmington, DE). Applications were initiated once severe TSSM populations had colonized the plant. Foliar treatments for all years tested were applied a 2 nozzle, 3-liter carbon dioxide hand held sprayer calibrated to deliver 93.54 liters per hectare (L ha^{-1}) with two Teejet TX-6 hollow cone nozzles (Teejet Technologies Glendale Heights, IL). Treatments consisted of glufosinate ammonium applied at 0.73, 1.61 and 3.14 L ha^{-1} and fenpyroximate at 1.17 L ha^{-1} for all years tested.

Leaf samples, for both studies, consisted of ten fully expanded leaves randomly pulled from the middle two rows of each plot in 2015 top 5 nodes 0, 5 and 14 days after application. Samples were placed in #2 hardware paper bags (Uline, Pleasant Prairie, WI). Whole leaves were processed using a mite brushing machine, (Model 2836M, Bioquip Products, Rancho Dominguez, CA) adult and immature mites were counted using a dissecting microscope and pooled for analysis.

Leaf Dip Bioassay

Research was conducted at the LSU AgCenter's MRRS in 2015. Seven concentrations of formulated Liberty 280 SL herbicide (0, 1, 5, 10, 15, 20 and 25 ppm) were obtained from serial dilutions and each concentration was replicated 8 times. Fifty-six healthy, arthropod free cotton leaves were collected from Stoneville 5289 GTL reared in the greenhouse at the Macon Ridge Research Station for leaf dip assays. Collected leaves were washed with tap water and placed abaxial side up and allowed to air dry for 1 hour. Once all moisture was dried from leaves, 8

leaves were randomly assigned to each treatment. Leaves were fully submerged in each concentration for 5 seconds, placed abaxial side up and allowed to air dry until all moisture has dissipated. A 2.54-cm punch was used to extract 8 leaf cores for each treatment. Individual leaf cores were placed in petri dishes filled with 15 ml of agarose gel. After the cores were placed on the gel surface, 10 female, field collected adult TSSM were placed on each core and each Petri dish was capped and paraffin applied to seal all gaps. Sealed petri dishes were placed in a growth chamber set to 27 °C with 75 % RH and 14:10 L:D setting. Spider mite mortality was assessed 48 hours after infestation. Mites were examined under a dissecting microscope and considered dead when mites failed to respond to prodding with a fine camel hair paint brush.

Spider mites from the foliar tests were subjected to a Henderson-Tilton transformation to calculate percent control taking into account the differences control and treatment changes from the time of treatment to the time of assessment (Henderson and Tilton 1955). Foliar data were subjected to ANOVA and means were separated using an F protected LSD ($P < 0.05$) (SAS Institute, 2010). Bioassay data were subjected to non-linear regression analysis and with 95% confidence intervals (CI) obtained for the TSSM population (Systat Software 2008). Mite mortality at each concentration was corrected based on the control mortality using the method of Abbott (1925). The regression line was constrained to force $y_0 = 0$ at x_0 . Regression analyses were tested for assumptions of linearity using the Spearman rank correlation between the absolute values of the residuals and the observed value of the dependent variable, normality was tested using Saprio-Wilk's test ($P < 0.05$), and outliers were detected and eliminated based on Studentized residuals, and disproportional influence using DFFTTs, Leverage and Cook's distance tests (SigmaPlot 12: User's Guide, 2010).

RESULTS

Foliar efficacy

Spider mite populations built up to damaging levels in 2015 while excessive precipitation prevented field efficacy studies in 2016 which were simulated in the greenhouse. Glufosinate ammonium applied at 0.73 and 1.61 L ha⁻¹ provided unsatisfactory control of TSSM relative to fenpyroximate in the 2015 field study (Table 4.1). At 5 days after application (DAA), glufosinate ammonium provided 45.66 percent control of TSSM and was not determined to be significantly different than the non-treated control. Glufosinate ammonium applied at 1.61 and 3.14 L ha⁻¹ provided 48.86 and 80.22 percent control while fenpyroximate provided 89.62 percent control 5 DAA. Applications of glufosinate ammonium 14 DAA provided from 3.19 to 54.19 percent control of TSSM, while fenpyroximate provided 69.41 percent control. Glufosinate ammonium applied at 3.14 L ha⁻¹ was determined to not be significantly different than the dedicated acaricide fenpyroximate at 5 and 14 DAA.

Significant phytotoxic effects were observed at the conclusion of this study. Glufosinate ammonium applied at 0.73 and 1.61 L ha⁻¹ caused between 15 and 25 percent chlorosis and necrosis of treated plots (Figure 4.1). No significant differences in phytotoxicity were detected between the 0.73 and 1.61 L ha⁻¹ rates. Glufosinate ammonium applied at 3.14 L ha⁻¹ caused significantly more phytotoxicity than any other treatment with 50 percent of treated plots experiencing substantial chlorotic and necrotic injury (Figure 4.1). Fenpyroximate and the non-treated check exhibited almost no phytotoxic (< 5%) symptoms resulting in no significant differences between treatments. Visible symptoms did not appear until after the study was concluded.

Table 4.1 Efficacy of glufosinate ammonium and fenpyroximate to field populations of TSSM in 2015.

| Treatment | Rate amount product(L ha ⁻¹) | Number of TSSM per 10 leaves | | | | | | | | | | |
|-----------------|---|------------------------------|--------|--------|----------------|--------|--------|--------------------|-----------------|---------|---------|--------------------|
| | | 13 Aug (pre-treatment) | | | 18 Aug (5 DAT) | | | | 27 Aug (14 DAT) | | | |
| | | adult | imm | motile | adult | imm | motile | % chk ^z | adult | imm | motile | % chk ^z |
| Non-treated | — | 85.6a | 136.7a | 232.3a | 328.4a | 213.6a | 552.4a | 0.00c | 461.3a | 753.3a | 1249.7a | 0.00c |
| glufosinate amm | 0.73 | 43.3a | 27.2a | 74.8a | 40.4b | 33.5bc | 76.8bc | 45.66c | 272.5ab | 282.1ab | 561.1ab | 3.19c |
| glufosinate amm | 1.61 | 98.6a | 36.1a | 135.4a | 61.0b | 91.5ab | 153.1b | 48.86bc | 177.8bc | 232.8b | 414.6b | 33.54b |
| glufosinate amm | 3.14 | 64.7a | 56.2a | 127.6a | 19.4b | 18.1c | 41.3c | 80.22ab | 101.5c | 125.3bc | 244.6bc | 54.19ab |
| fenpyroximate | 1.71 | 42.3a | 83.8a | 129.9a | 16.5b | 13.4c | 31.5c | 89.62a | 99.3c | 65.8c | 165.7c | 69.41a |

Values in a column followed by the same letter are not different based on ANOVA and a protected LSD ($P \leq 0.05$).

^zPercent of non-treated control (Henderon-Tilton) of foliar applications on TSSM populations.

Table 4.2 Efficacy of glufosinate ammonium and fenpyroximate to greenhouse populations of TSSM in 2016.

| Treatment | Rate amount product (L ha ⁻¹) | Number of TSSM per 10 leaves | | | | | | | | | | |
|-----------------|--|------------------------------|-------|--------|----------------|-------|--------|--------------------|-----------------|-------|--------|--------------------|
| | | 7 Dec (pre-treatment) | | | 12 Dec (5 DAT) | | | | 21 Dec (14 DAT) | | | |
| | | adult | imm | motile | adult | imm | motile | % chk ^z | adult | imm | motile | % chk ^z |
| Non-treated | — | 20.6a | 41.3a | 89.8a | 45.0a | 61.8a | 106.8a | 0.00c | 54.8a | 79.5a | 134.3a | 0.00d |
| glufosinate amm | 0.73 | 22.3a | 21.0a | 64.8a | 25.0a | 11.5b | 36.5b | 55.50b | 26.3a | 19.8b | 46.0b | 55.43c |
| glufosinate amm | 1.61 | 42.4a | 41.8a | 102.8a | 33.5a | 17.8b | 51.3b | 57.36b | 25.3a | 12.8b | 38.0b | 72.86bc |
| glufosinate amm | 3.14 | 77.7a | 52.2a | 137.3a | 16.3a | 12.8b | 29.0b | 80.62ab | 8.3a | 6.3b | 14.5b | 91.85ab |
| fenpyroximate | 1.71 | 24.0a | 42.5a | 102.0a | 6.8a | 5.5b | 12.3b | 89.35a | 2.3a | 2.0b | 4.3b | 96.88a |

Values in a column followed by the same letter are not different based on ANOVA and a protected LSD ($P \leq 0.05$).

^zPercent of non-treated control (Henderon-Tilton) of foliar applications on TSSM populations.

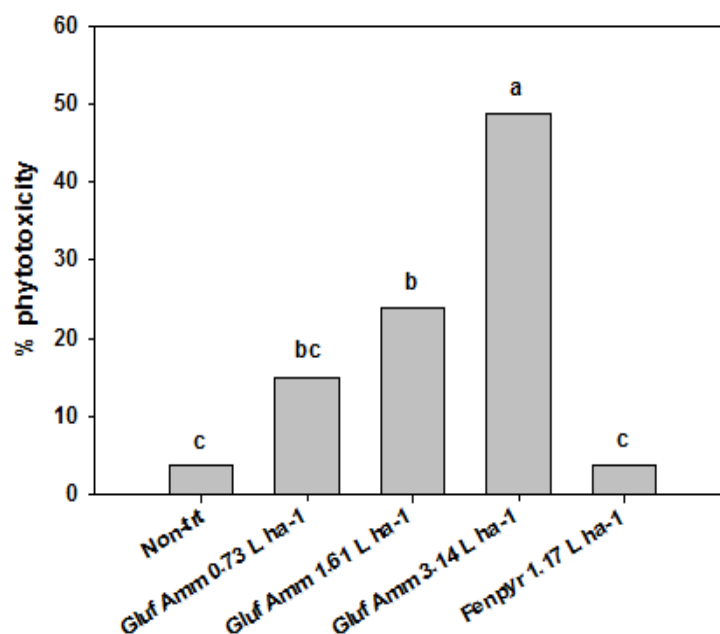


Figure 4.1 Phytotoxicity ratings of foliar applications in 2015. Columns containing the same letter are not significantly different based on ANOVA and an F protected LSD ($P \leq 0.05$).

For the 2016 greenhouse study, glufosinate ammonium was determined to be efficacious at all rates tested 5 DAA with only the 3.14 L ha⁻¹ rate reducing TSSM populations equal to fenpyroximate at both 5 and 14 DAA (Table 4.2). Total motiles in all treatments except the non-treated control were not significantly different based on ANOVA and a protected LSD ($P \leq 0.05$) 5 DAA. Glufosinate ammonium applied at 0.73 L ha⁻¹ resulted in 55.43 percent control 14 DAA while 1.61 L ha⁻¹ resulted in 72.86 percent control and 3.14 L ha⁻¹ resulted in 91.85 percent control of TSSM populations. Fenpyroximate provided the greatest control of all years tested.

Leaf dip bioassay

Leaf dip bioassay results indicated that TSSM were highly susceptible to concentrations of formulated glufosinate ammonium. The LC₅₀ value was determined to be 10.31 ppm with 95% CI determined to be (6.02 – 15.81) (Figure 4.2). Non-linear regression analysis indicated a significant ($P < 0.0001$) dose mortality relationship ($R^2 = 0.48$).

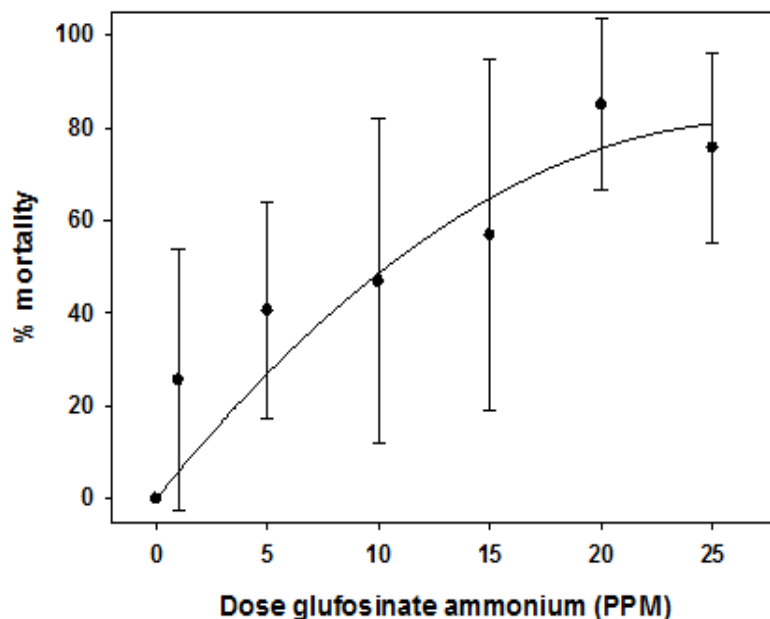


Figure 4.2 Dose response curve of TSSM to glufosinate ammonium in leaf dip bioassays. $R^2 = 0.48$, $F(2, 54) = 49.65$, $P < 0.0001$.

DISCUSSION

The use of glufosinate ammonium, on damaging populations of TSSM, provided control comparable to a standard acaricide when used at the maximum label rate in the field. Dose mortality bioassays indicated that TSSM were highly susceptible to glufosinate ammonium and appropriate field use rates may provide an added acaricidal benefit to pre-plant weed management or post emergence use during the recommended label use window. However, use of glufosinate ammonium at the stage conducted in the 2015 experiment would be considered an off-label application. Glufosinate ammonium requires a 70 day pre-harvest interval (PHI) which allows for foliar applications to be made in the early squaring to first bloom period. Furthermore, the high cost associated with the use of this herbicide is not considered a viable treatment targeting spider mites. Glufosinate ammonium formulated as Liberty 280 SL herbicide would cost producers \$54.00 per hectare when applied at 0.73 L ha^{-1} and \$106.00 per hectare when applied at 3.14 L ha^{-1} while fenpyroximate formulated as Portal XLO costs \$30.00 per hectare.

Dedicated acaricides such as fenpyroximate are significantly less expensive (\$22.00 – 30.00 per hectare), have shorter PHI's and cause very little phytotoxicity when used appropriately. The cotton utilized for this test was experiencing severe drought stress, coupled with advanced maturity resulted in the abnormal levels of phytotoxicity experienced.

However, the use of glufosinate ammonium as an alternative form of mite control may be a highly effective tool for managing TSSM populations resistant to traditional acaricides. Ahn et al. (1997) demonstrated efficacy of glufosinate ammonium to TSSM field populations highly resistant to various acaricides. Thus, the acaricidal mode of action of glufosinate ammonium may be different from that of known compounds, although the exact mechanism remains unknown. Furthermore, Ahn et al. (1997) also demonstrated a positive temperature coefficient for glufosinate ammonium (10 to 32°C) on TSSM mortality when applied by the mite dipping method. Glufosinate ammonium toxicity was shown to increase 17 and 20 times that at 10°C when temperatures were elevated to 25 and 32°C (Ahn et al. 1997). This may further help elucidate a possible mechanism of action of glufosinate ammonium but may also have other implications for mite control as well. The use of glufosinate ammonium as a pre-plant herbicide may impart only partial acaricidal benefits if the weather is cool. Louisiana has an average spring temperature of 19°C while the average summer temperature is 27°C, spring pre-plant herbicide applications are made in spring while squaring and bloom applications are often made during the summer (NCDC 2015). Applications of glufosinate ammonium during spring months may only suppress TSSM populations while applications made during summer months may offer more adequate control of TSSM populations. Additionally, glufosinate ammonium does not exhibit any repellency properties that may cause mite movement to non-affected weeds or refuges where further feeding and reproduction would result in further outbreaks and was also determined to be

relatively non-toxic to non-target arthropods including beneficial insects and mites (Ahn et al. 2001). They found that glufosinate ammonium applied at 540 ppm (field applied rate for weed control in apples) was determined to be non-toxic to eggs of *Amblyseius womersleyi* Schicha, *Phytoseiulus persimilis* Athias-Henriot, and *T. urticae* but acutely toxic to nymphs and adults. Experiments with *Chrysopa pallens* Rambur demonstrated little or no harm to larvae and pupae, while mortality of *Orius strigicollis* Poppius was determined to be 71.2% to eggs, 65.0% to nymphs and 57.7% to adults at 540 ppm. Overall, glufosinate ammonium is less toxic to beneficial insects with the exception of the predatory mite *P. persimilis* (Ahn et al. 2001).

In conclusion, glufosinate ammonium may be a key component of integrated pest management for TSSM control in cotton. The use of glufosinate ammonium as a resistance management tool, for glyphosate resistant weeds such as palmer amaranth coupled with the acaricidal benefits demonstrated in this study, may give producers an effective option in controlling weeds as well as populations of spider mites in cotton. Additionally, the effects of glufosinate ammonium on TSSM populations in soybeans and corn as well as further investigations into this compounds mode of action are warranted.

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CHAPTER 5: SUMMARY AND CONCLUSIONS

The TSSM, *Tetranychus urticae* (Koch), status as an economic pest in Midsouth cotton has changed over the last 10 years. Historically, spider mites have been considered a late-season pest in the Midsouth with pesticide applications often rarely needed during early reproductive stages of cotton development. However, spider mites have become an increasing problem in recent years. Numerous factors such as the use of neonicotinoid based insecticide seed treatments, use of broad-spectrum insecticides for control of other economically important pests, and inadequate or poor fall and spring vegetation management may have contributed to the increase in spider mites becoming a season-long pest in Midsouth cotton production systems. Further adding to this issue is, the TSSM's propensity for rapidly developing resistance to insecticides and acaricides. The risks of resistance in TSSM is enhanced by its arrhenotokous reproduction, high fecundity, short life cycle, and propensity for inbreeding. These aspects have led to the TSSM being considered the 'most resistant' in terms of total number of pesticides to which populations have become resistant. The acaricide abamectin has been extensively used for control of TSSM in cotton over the past decade in the Midsouth. Recently, growers have observed reduced efficacy and shortened residual control, indicating a possible issue with resistance development. Independent of seed treatments and acaricide use, the practice of controlling vegetation prior to planting and during the growing season eliminates "the green bridge" from which pest arthropods migrate into agricultural fields. An example of this is the use of LL cotton to combat glyphosate resistant weed species. The use of glufosinate ammonium herbicide for burndown applications and in crop use may provide agricultural producers with a secondary acaricidal benefit when TSSM are present on weeds and field crops.

Currently, there is limited information available to address these concerns. Therefore, multiple field and laboratory tests were designed to: 1) determine the effects of foliar applied jasmonic acid and seed applied imidacloprid on phytohormone expression and TSSM populations in cotton; 2) determine baseline toxicity of abamectin to Louisiana and Midsouth populations of TSSM; 3) evaluate the efficacy and toxicity of glufosinate ammonium on populations of TSSM; 4) measure reproduction and fecundity of TSSM on imidacloprid and jasmonic acid treated cotton; and 5) determine the effects of foliar applied jasmonic acid and seed applied imidacloprid on the infestation preference of TSSM.

During the 2013 field season, imidacloprid seed treatments significantly increased cumulative adult mite days; however, no measurable differences were determined during 2015 or 2016. Applications of 10 millimolar jasmonic acid did not reduce mite severity or injury in all field trials. Imidacloprid seed treatments significantly increased all spider mite life stages in the laboratory while applications of jasmonic acid significantly reduced all mite life stages on neonicotinoid treated and non-treated cotton. Seed treatments do not affect the host preference of twospotted spider mites compared to non-treated however, jasmonic acid applications reduced the host suitability of seedling cotton to only adult mites. Results from this study highlight the unintended consequences of using seed treatments for early season insect control in cotton. However, their use is vital to protecting seedling cotton from insects such as thrips and wireworms. Furthermore, our results documented the possible use of exogenous applications of JA as novel plant protection compound for arthropods in cotton.

Multiple leaf-dip bioassays were conducted in 2013, 2014 and 2015 to determine if populations of TSSM from the Midsouth exhibited resistance to abamectin. Based on our findings, two populations from Louisiana were documented to possess the highest levels of

resistance to abamectin with corresponding LC_{50} values of 0.082 and 0.184 ppm and resistance ratios of 630 and 1415-fold. While one population from Mississippi was slightly resistant with an LC_{50} value of 0.0021 ppm and a resistance ratio of 11.1 compared with a susceptible control population. LC_{50} values for all colonies were significantly greater than the control population. These results demonstrate that variable levels of abamectin resistance exists in populations of TSSM from Louisiana, Mississippi, Arkansas, and Tennessee. Implications from this study emphasize the importance of implementing integrated pest management and judicious use of acaricides for control of damaging populations of TSSM.

During 2015-2016, multiple foliar trials and laboratory bioassays were conducted using glufosinate ammonium to assess the susceptibility of TSSM in LL cotton. The results demonstrated that field applied glufosinate ammonium at 1.61 and 3.14 L ha⁻¹ provided 48.86 and 80.22 percent control while fenpyroximate provided 89.62 percent control 5 days after application in 2015. Greenhouse applications resulted in 55.43 percent control 14 days after application with 0.73 L ha⁻¹ while 1.61 L ha⁻¹ resulted in 72.86 percent control and 3.14 L ha⁻¹ resulted in 91.85 percent control of TSSM populations. Treatment with glufosinate ammonium resulted in significant phytotoxic effects to drought stressed cotton in the 2015 field trial. While leaf dip bioassay results indicated that TSSM were highly susceptible to concentrations of formulated glufosinate ammonium. The LC_{50} value was determined to be 10.31 ppm. These results suggest that glufosinate ammonium may be useful tool for integrated pest management of weeds and spider mites in cotton. Due to the high cost associated with glufosinate ammonium and possibility of phytotoxic effects under certain conditions, this herbicide it is not considered a viable treatment targeting spider mites but may prove useful for controlling mites when utilized for weed management.

APPENDIX: LETTERS OF PERMISSION.

Letter of Permission for Chapter 2

RE: Sebe Brown, David L. Kerns, Jeffrey Gore, Gus Lorenz, Scott Stewart, Susceptibility of twospotted spider mites (*Tetranychus urticae*) to abamectin in Midsouth cotton, *Crop Protection*, Volume 98, August 2017, Pages 179-183, ISSN 0261-2194, <http://doi.org/10.1016/j.cropro.2017.04.002>.

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VITA

Sebe Anthony Brown was born in Beaumont, TX. After graduating from high school, he attended Texas A&M University in College Station, TX where he received the degree of Bachelor of Science in Entomology in 2009. After graduation, Sebe enrolled into the graduate program in the department of Entomology at Louisiana State University from 2010-2012, where he received the degree of Master of Science in Entomology. His master's research focused on evaluating the efficacy of methoxyfenozide on Louisiana, Texas and the Mid-Southern soybean looper populations. In January, 2013, Sebe began his doctoral studies under Dr. David Kerns in the Department of Entomology at Louisiana State University. Currently he is a doctoral candidate in the Department of Entomology. His dissertation research is focused on the integrated pest management of twospotted spider mites in cotton. He is currently completing the requirements for the degree of Doctor of Philosophy and plans to pursue a career in applied insect pest management research and extension. Sebe is married to Kimberly Pope Brown.