ABSTRACT

KOMAR, TABATHA ANN. Integration of Novel Insecticide Tools into Contemporary Cotton IPM Systems (Under the direction of Dr. Anders Huseth).

Cotton (*Gossypium* spp.) is infested by many insect pests which results in large annual insecticidal inputs into the crop. To decrease pest pressure below economically damaging levels, cotton pest management has relied on broad-spectrum insecticides that have detrimental impacts on human and environmental health. Over time, more selective active ingredients, and genetically engineered plants with plant-incorporated-protectants from *Bacillus thuringiensis* (*Bt*) have changed the insecticidal footprint of the cotton system. However, given the widespread adoption of genetically engineered crops, secondary pest outbreaks and insecticidal resistance is an ongoing problem, often necessitating foliar insecticide use. The overarching goal of this project is to document how pest management innovations may change cotton Integrated Pest Management (IPM).

In my first study, I examined the efficacy of the new trait expressed by ThryvOn™ (developed by Bayer Crop Science); ThryvOn, which expresses Cry51Aa2.834_16, is the first genetically engineered toxin to target piercing-sucking thysanopteran and hemipteran pests. The study aimed to collect baseline mortality and reproductive fitness data for tobacco thrips (*Frankliniella fusca* Hinds) and western flower thrips (*Frankliniella occidentalis* Pergande). This was accomplished by assaying two laboratory colonies and nine field-collected populations from three distinct cotton growing regions in the United States (Southeast, Mid-south, and West). Thrips oviposition and mortality was monitored for 6-days on either near isoline (non-*Bt*) plant tissue or the ThryvOn trait. Despite limited mortality, our study showed significant oviposition

deterrence in all populations. These results support ongoing ThryvOn field trials and the generation of baseline efficacy data that will inform resistance monitoring efforts in the future.

The second study focused on the impacts of foliar applications on natural enemies in cotton agroecosystems. In this multiyear field study across multiple states, we applied common lepidopteran and hemipteran insecticides and tracked changes in natural enemy community composition that resulted from insecticide exposure. We also focused on two important generalist predators: *Geocoris* spp. and *Orius insidiosus* (Say), given their facultative herbivory and similarities to a target pest, *Lygus lineolaris*. Results showed that natural enemy communities exposed to methoxyfenozide, chlorantraniliprole, sufloxaflor, novaluron, spinetoram, and bifenthrin were not significantly impacted. However, our results showed a decrease in natural enemy abundance with the broad-spectrum insecticide acephate, supporting prior work documenting the negative effects of broad-spectrum organophosphate insecticides on natural enemies. Overall, the study showed natural enemy communities were relatively resilient to newer modes of action but clearly highlights the impact that broad-spectrum insecticides have on natural enemy communities.

Integration of Novel Insecticide Tools into Contemporary Cotton IPM Systems

by
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DEDICATION

To my parents, family, friends and husband for the kind words of encouragement on days I needed it most.

BIOGRAPHY

Tabatha Komar grew up in a small rural town in Wisconsin. In her own backyard, she was able to witness the intersection of wetlands and agriculture. She always had an interest in nature, so she pursued a major in biology and a minor in chemistry at the University of Wisconsin Eau Claire. Sitting through conservation and entomology courses, she realized that entomology was her passion, especially from a standpoint of preservation and conservation. After witnessing how agriculture dominates the landscape in the Midwest, Tabatha wanted to pursue a career in agricultural entomology to be a part of the research that better informs sustainable agroecosystems. She was fortunate enough to hold a position at Syngenta where she gained experience in the industrial side of pest management. Tabatha continued on to pursue her Master's degree in Entomology at North Carolina State University to further her education and gain greater experience in ecology and entomology.

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CHAPTER 1

A review of novel decisions in pest management and their impacts on cotton agroecosystems

Problem Defined

Project Overview and Literature Review Goal: This project focuses on the impact of pest management changes to the cotton production system. Specifically, I identified two key changes to study 1) characterizing the susceptibility of a thrips species to a novel *Bacillus thuringiensis* toxin (Cry51Aa2.834_16; marketed as ThryvOn, Bayer Crop Science) expressed in cotton; and 2) documenting the non-target effects of foliar insecticides on natural enemies commonly found in cotton. Together, these two objectives help to define a foundation for future pest management research that explore the impact of specific pest management changes in the cotton system. The goal of this literature review is to describe the current production system and to provide context for the questions addressed in Chapters Two and Three of this thesis.

The Current State of Cotton Production in the United States

Cotton (*Gossypium* spp.) is one of the most important fiber commodities worldwide with trade of both raw and unfinished products (USDA ERS, 2020a). The United States is the third largest cotton producer, accounting for roughly 17.6 million bales per year, of which is comprised of 97% upland cotton (*Gossypium hirsutum* L.) (USDA ERS 2020; USDA FAS, 2022). Cotton is grown by farmers in the United States at an estimated 10.4 to 12.1 million acres valued at more than $7 billion (USDA, 2021; USDA ERS, 2020). For the past several decades demand for cotton has increased, driven by consumer demand for naturally produced fiber and rising petroleum prices that increase costs for synthetic textiles (USDA ARS, 2021).

Cotton is produced across 17 southern states from California to Virginia, commonly referred to as the "Cotton Belt" with Texas being the largest producer in area and total yield (USDA ERS, 2020). Cotton ranks as one of the top cash crops available to grow in the Mid-South; however, it requires a consistent moderate temperature that is also favorable for many agricultural pests (Bottrell & Adkisson, 1977; USDA NASS, 2017).

History of Pest Management in Cotton

Cotton attracts many arthropod herbivores due to the variety of reproductive and vegetative tissues available at a given time. Previous research reported that there are more than one thousand species of insects and spider mites that can feed on cotton; however, approximately a dozen species cause consistent crop loss if left unmanaged (Deguine *et al.,* 2007). One challenge for cotton management is sequential infestations from different arthropod pests during the season, this repeated infestation leads to a large annual insecticidal input into the crop (Luttrell *et al.,* 1994; Deguine *et al.,* 2008). Following World War II, synthetic insecticides targeting key lepidopteran and coleopteran pests became a common component of cotton production during the late 1940s and 1950s (Casida and Quistad, 1998; Kennedy, 2008). During this era, deemed the Golden Age of Insecticide Discovery, entomologists became attracted to the efficacy that synthetic insecticides provided and focused on new improvements for insecticidal chemicals, mainly organochlorines and organophosphates. In the 1980s, carbamates and pyrethroids also grew in popularity (Casida and Quistad, 1998). Following the publication of Silent Spring, risks and concerns emerged about the overreliance of synthetic insecticides largely due to environmental persistency and cascading impacts on ecosystems and human health (Carson, 1962; Coats, 1990). The Pesticide Control Act was enacted in 1972, after the environmental fallout from bioaccumulation accredited to the widespread use of the

organochloride, DDT (Pesticide Control Act, 1972). The act enforces government restriction of certain toxic compounds and regulates their usage (Pesticide Control Act, 1972). As regulations became stricter, chemical companies sought less toxic and persistent compounds that would have fewer non-target impacts. Higher potency of chemicals used against arthropods has historically led to a decrease in the total amount of active ingredient used (Ruberson *et al.,* 1998). Unfortunately, occurrence of pesticide resistance in cotton pests became common over time, and this process continues to threaten the sustainability of chemical pest management (Palumbo *et al.,* 2001; Huseth *et al.,* 2016; Huseth *et al.,* 2018; Reisig *et al.,* 2018a & b). Cotton pest management strategies in the last 50 years in cotton have had to be developed to combat several secondary pest outbreaks, resurgences of pest populations, and ecological impacts associated with widespread adoption of broad-spectrum synthetic insecticidal chemicals (Deguine *et al.,* 2007; Kennedy, 2008).

Adoption of Insecticidal Genetically Engineered Cotton

One of the most pivotal changes in the history of pest management was the development of genetically engineered (GE) crops that express insecticidal toxins or have herbicide tolerant traits (Fernandez-Cornejo *et al.,* 2006; Mall *et al.,* 2018). This turning point increased crop productivity by providing in-plant protection against insects and enabled the use of herbicides without harming crops.

In 1987, the first insect-resistant GE plants were developed to express modified *Bacillus thuringiensis* (*Bt*) proteins as plant-incorporated-protectants (PIPs) to protect plants against lepidopteran pests (Vaeck *et al.,* 1987; Jurat-Fuentes *et al.,* 2021). Crystal protein genes (Cry) were the first insecticidal toxins added to GE cotton; these toxins encode crystalline proteins in plant tissues that, when consumed by lepidopteran larvae, damage the gut lining and result in

mortality (Höfte and Whiteley, 1989; Orford *et al.*, 2007). In 1996, Bollgard cotton that expresses Cry1Ac *Bt* toxin was commercialized for control of tobacco budworm (*Chloridea virescens* Fabricius), pink bollworm (*Pectinophora gossypiella* Saunders), and bollworm (*Helicoverpa zea* Boddie) (Kennedy, 2008; Sivasupramaniam *et al.*, 2014). Bollgard cotton was an immense step forward for cotton sustainability because the technology displaced synthetic insecticides that had known non-target effects on beneficial insects and the surrounding environment (Graham, 2005; Orford *et al.*, 2007; Kennedy, 2008; Krishna *et al.*, 2012; Fernandez-Cornejo *et al.*, 2014; Gowda *et al.*, 2016). After the initial release of *Bt* cotton, the U.S. Environmental Protection Agency (EPA) documented significant insecticide use reductions in most cotton growing regions of the southern United States (Shelton *et al.*, 2002).

The adoption of GE crops has improved agricultural economy and crop sustainability by decreasing pesticide use while increasing yields (Shelton *et al.*, 2002; Huang *et al.*, 2003; Kennedy, 2008). The integration of GE technology also improves Integrated Pest Management due to the selective nature of current *Bt* toxins and the relatively low risk to non-target arthropods (Naranjo *et al.*, 2005; Kennedy, 2008). The area-wide suppression of lepidopteran pests and preservation of beneficial insect communities made GE crops popular across many countries and provides a powerful tool for sustainable pest management (Hutchinson *et al.*, 2010; Tabashnik and Carriere, 2017; Dively *et al.*, 2018).

Current challenges for GE cotton pest management

Thrips as a Persistent Cotton Pest: Current and Future Management Tools

Several species of thrips (Thysanoptera) are pests of seedling cotton, including but not limited to, western flower thrips (*Frankliniella occidentalis* Pergande), tobacco thrips (*Frankliniella fusca* Hinds), and onion thrips (*Thrips tabaci* Lindeman) (Watts, 1937; Sadras and

Wilson, 1998; Cook *et al.,* 2011). Early season thrips injury to cotton plants occurs from emergence through 4-5 true leaves and can reduce plant health (Cook *et al.,* 2011). Thrips feed on the epidermal cells of the cotyledons and true leaves of the cotton seedling (Quisenberry and Rummel, 1979; Cook *et al.,* 2011). Cells are ruptured and drained of fluids, causing a silvery appearance on the plant tissue (Cook *et al.,* 2011). Areas with injury do not grow normally; causing the leaves to twist or curl and, as a result, reduce photosynthetic capacity (Wene *et al.,* 1965; Quisenberry, and Rummel, 1979; Cook *et al.,* 2011). Under heavy pressure, thrips feeding can reduce plant height and growth, and delay crop flowering and subsequent fruiting (Watts, 1937; Leigh, 1963; Race, 1965; Wene *et al.,* 1965). Prolonged time to fruiting can result in later maturation and delayed boll development of cotton crops, which can delay harvest (Watts, 1937; Cook *et al.,* 2011). For several decades, thrips have been intensively managed using in-furrow, seed treatment, and foliar insecticides (Cook *et al.,* 2011). However, studies of thrips as a pest of cotton do not always find a consistent negative relationship between infestation intensity and yield (Watts, 1937; Leigh, 1963; Race 1965; Rummel *et al.,* 1979; McNaughton, 1983; Wilson *et al.,* 1996; Sadras and Wilson 1998; Cook *et al.,* 2011). Despite an inconsistent yield and infestation relationship, thrips are still prophylactically treated with systemic seed treatments or in-furrow applications on the majority of U.S. cotton (Akin *et al.,* 2010; Douglas and Tooker, 2015).

Current recommendations for early-season thrips control rely on systemic neonicotinoid insecticides that limit larval establishment on the plant and therefore prevent damage (Elbert *et al.,* 2008; Roberts *et al.,* 2012; Herbert *et al.,* 2021). Systemic neonicotinoid (thiamethoxam or imidacloprid) seed treatments target *Frankliniella fusca*, the most prevalent thrips species in the eastern part of the U.S. Cotton Belt and have replaced older materials, such as aldicarb (Temik®;

Bayer CropScience, Research Triangle Park, NC) (Maienfisch *et al.*, 2001; Elbert *et al.*, 2008; Cook *et al.*, 2011). During the last decade, the evolution of neonicotinoid resistance in *F. fusca* populations has driven widespread increases in foliar and in-furrow insecticide inputs to control problematic populations and reduce probability of damage across the Cotton Belt (Huseth *et al.*, 2016; Huseth *et al.*, 2018; Darnell-Crumpton *et al*. 2018).

In response to emerging control issues for piercing-sucking cotton pests, the first GE trait targeting thysanopteran and hemipteran in cotton has been commercialized by Bayer Crop Science (St. Louis, MO). This novel GE technology, marketed as ThryvOn™, expresses a modified Cry51Aa2 insecticidal protein that has activity against hemipteran and thysanopteran pests (Bachman *et al.*, 2017; Akbar *et al.*, 2019). Because plant bugs (*Lygus* spp.) and thrips are an important pest group in cotton, the commercialization of this pest management tool could improve the sustainability of cotton production by reducing reliance on synthetic insecticides for control of this pest complex (Cook & Threet, 2020; Akbar *et al.*, 2019). Additionally, ThryvOn may provide flexibility to manage emerging resistance in both pests (Darnell *et al.*, 2015; Huseth *et al.*, 2016; Dorman *et al.*, 2020).

After several laboratory and field studies of ThryvOn, it is apparent that this technology has a unique mechanism of activity against target pests. The technology does not appear to be acutely toxic for either *Lygus lineolaris* or *Frankliniella* spp., but instead reduces pest populations through a combination of non-lethal reproductive and developmental effects (Bachman *et al.*, 2017; Akbar *et al.*, 2018; Graham *et al.*, 2019; Huseth *et al.*, 2020; D'Ambrosio *et al.*, 2020a & b). Specifically, thrips populations are suppressed through behavioral avoidance, reduced oviposition and antifeedant effects (Huseth *et al.*, 2020; D'Ambrosio *et al.*, 2020a & b). While the technology broadens the spectrum of pest control with *Bt* toxins, it is not a high dose

against susceptible pests, a benchmark that regulatory agencies typically request, which may further complicate resistance management (Caprio, 2000; Graham *et al.,* 2019; Huseth *et al.,* 2020; D'Ambrosio *et al.,* 2020a & b).

An emerging challenge for *Bt* cotton: secondary pest outbreaks complicates biological control services

While *Bt* technology provided significant benefits for pest control, secondary pest outbreaks have generated new concerns about the long-term sustainability of the agricultural production systems dominated by GE crops (Krishna *et al.,* 2012; Gutierrez, 2005; Catarino *et al.,* 2015). The decrease in insecticides targeting lepidopteran pests and boll weevil has been linked to the emergence of secondary pests, particularly hemipterans, that were previously controlled by insecticides targeting key lepidopteran pests (Gutierrez, 2005; Lu *et al.,* 2011; Zeilinger *et al.,* 2015). In the United States, the *Lygus* spp. has become a key concern in *Bt* cotton across the Cotton Belt (Ellsworth and Barkley, 2005; Dorman *et al.,* 2020). Similar secondary outbreaks of plant bugs (Hemiptera: Miridae) in China and stink bugs (*Nezara viridula* L.) in Australia have driven and increase in foliar insecticide applications, undermining some of the positive benefits of *Bt* cotton (Lu *et al.,* 2011; Wilson *et al.,* 2013). In India, mirids, mealybugs (Hemiptera: Pseudococcidae), and other sucking pest species not controlled by *Bt* are changing attitudes about the value of *Bt* cotton (Krishna and Qaim, 2012). These outbreaks have been particularly challenging for developing countries where the cost of foliar insecticidal applications for secondary pests in addition to the cost of *Bt* cotton seed can have significant impacts on farmer profits (Dowd-Uribe, 2014; Qiao, 2015).

Since the release of Bollgard *Bt* in 1996, the widespread adoption of *Bt* cotton and development of more selective pesticides provide advancements in the role of biological control

in cotton pest management in the United States, by reducing detrimental broad-spectrum pesticides (Shelton *et al.,* 2002; Head *et al.,* 2005). Newly created transgenic plants must partake in an environmental risk assessment to evaluate impacts on non-target organisms, where behavior, fitness and ecology are assessed (Andow and Zwahlen, 2006). There is a wealth of literature that explores tritrophic interactions between parasitoids, natural enemies, and their prey, feeding on transgenic crops (Schuler *et al.,* 1999; Schuler *et al.,* 2003; Lundgren *et al.,* 2005; Shahid *et al.,* 2019; Shelton *et al.,* 2016). In most published cases, tritrophic interactions resulted in no negative impacts on the evaluated natural enemies, implying that *Bt* toxins are not harmful against natural enemies (Schuler *et al.,* 2003; Lundgren, *et al.,* 2005; Shelton *et al.,* 2016) In a six-year study evaluating changes in diversity to non-target organisms from *Bt* crops, minimal declines were witnessed; however, the declines may be attributed to a loss of lepidopteran prey (Naranjo *et al.,* 2005). Furthermore, certain experiments and genetic modeling show that the presence of natural enemies may delay resistance in crop pests (Schuler *et al.,* 1999; Liu *et al.,* 2014). Natural enemies are becoming increasingly important for control of secondary pest outbreaks (Gross and Rosenheim, 2011; Tian *et al.,* 2015).

However, there are concerns with omnivorous hemipteran predators that may be negatively affected by ThryvOn during plant feeding stages. During characterization of the activity spectrum of Cry51Aa2.834_16, it was reported that there was a significant negative impact on survival of the insidious flower bug (*Orius insidiosus* Say; Hemiptera, Anthocoridae) (Bachman *et al.,* 2017). This is important because *Orius insidiosus* is an important key biological control agent for whitefly, *Heliothine* eggs and larvae, and spider mites (Elkassabany, 1994; Coll *et al.,* 1995). When ThryvOn is deployed in 2023, analyses should identify impacts on biological

control services rendered by beneficial non-target organisms to make the best suggestions for integrated pest management (Kim *et al.,* 2021).

Impact of Pesticides on Natural Enemies

As resistance issues arise, and secondary pest outbreaks continue to worsen, foliar insecticides are used regularly in cotton agroecosystems. The exposure to foliar pesticides may have variable impacts on natural enemies in agriculture; doses may be lethal and expressed as either acute or chronic (Ruberson *et al.,* 1998). As novel insecticides are licensed, they must undergo evaluation that assesses the toxicity; however, it does not consider any sub-lethal impacts (Sanchez-Bayo & Tennekes, 2017; Siviter & Muth, 2020). Sublethal effects can be more difficult to measure, but present themselves as shortened life-span, reduced fecundity, weight loss, or other detrimental physiological and behavioral changes (Ruberson *et al.,* 1998; Starks and Banks, 2003; Desneux, 2007). As insecticides continue to be deployed, the sublethal impacts that hinder biological control services may go undetected by regulatory processes (Siviter & Muth, 2017; Müller, 2018). This oversight may result in pest management decisions that do not work in tandem with natural enemies. While older insecticides, such as pyrethroids, organophosphates and neonicotinoids, have more well documented effects, recorded impacts from novel insecticides on natural enemy communities in field-applied settings and biological control services continue to be a dearth in informing integrated pest management (Desneaux *et al.,* 2007).

References

Akbar, W., Gowda, A., Ahrens, J. E., Stelzer, J. W., Brown, R. S., Bollman, S. L., ... & Clark, T. L. (2019). First transgenic trait for control of plant bugs and thrips in cotton. *Pest Management Science*, **75**(3), 867-877.

Akin, D. S., Lorenz, G. M., Studebaker, G. E., Stewart, S. D., Leonard, B. R., Micinski, S., ... & Bacheler, J. (2010). Evaluation of automatic insecticide applications following preventative insecticides for thrips: preliminary results from a regional project. In *Proceedings Beltwide Cotton Conference, New Orleans, LA* (pp. 4-7).

Andow, D. A., & Zwahlen, C. (2006). Assessing environmental risks of transgenic plants. *Ecology Letters*, **9**(2), 196-214.

Bachman, P.M., Ahmad, A., Ahrens, J.E., Akbar, W., Baum, J.A., Brown, S., Clark, T.L., Fridley, J.M., Gowda, A., Greenplate, J.T., Jensen, P.D., Mueller, G.M., Odegaard, M.L., Tan, J., Uffman, J.P., & Levine, S. L. (2017). Characterization of the activity spectrum of MON 88702 and the plant-incorporated protectant Cry51Aa2. 834_16. *PLoS One*, **12**(1), e0169409.

Caprio M.A., Sumerford D.V., & Sims S.R., (2000). Evaluating transgenic plants for suitability in pest and resistance management programs. *Field Manual of Techniques in Invertebrate Pathology* (ed. by L Lacey & H Kaya), pp. 769–792.

Carson, R. (1962). *Silent spring*. Boston: Houghton Mifflin

Casida, J. E., & Quistad, G. B. (1998). Golden age of insecticide research: past, present, or future?. *Annual Review of Entomology*, **43**(1), 1-16.

Coats, J. R. (1990). Mechanisms of toxic action and structure-activity relationships for organochlorine and synthetic pyrethroid insecticides. *Environmental Health Perspectives*, **87**, 255-262.

Coll, M., & Ridgway, R. L. (1995). Functional and numerical responses of Orius insidiosus (Heteroptera: Anthocoridae) to its prey in different vegetable crops. *Annals of the Entomological Society of America*, **88**(6), 732-738.

Cook, D., Herbert, A., Akin, D. S., & Reed, J. (2011). Biology, crop injury, and management of thrips (Thysanoptera: Thripidae) infesting cotton seedlings in the United States. *Journal of Integrated Pest Management*, **2**(2), B1-B9.

Cook, W., & Threet, M. (2020). Cotton insect losses [Online]. Mississippi State University. https://www.biochemistry.msstate.edu/resources/pdf/2020/table28.pdf [Accessed: 4 February 2022].

D'Ambrosio, D. A., Huseth, A. S., & Kennedy, G. G. (2018a). Temporal efficacy of neonicotinoid seed treatments against Frankliniella fusca on cotton. *Pest Management Science*, **74**(9), 2110-2115.

D'Ambrosio, D. A., Huseth, A. S., & Kennedy, G. G. (2018b). Evaluation of alternative mode of action insecticides in managing neonicotinoid-resistant Frankliniella fusca in cotton. *Crop Protection*, **113**, 56-63.

D'Ambrosio, D.A., Kennedy, G.G. & Huseth, A.S. (2020a). Feeding behavior of *Frankliniella fusca* on seedling cotton expressing Cry51Aa2.834_16 *Bt* toxin. *Pest Management Science,* **76,** 2781-2786.

D'Ambrosio, D. A., Kennedy, G. G., & Huseth, A. S. (2020b). Frankliniella fusca and Frankliniella occidentalis response to thrips-active Cry51Aa2. 834_16 *Bt* cotton with and without neonicotinoid seed treatment. *Crop Protection*, **129**, 105042.

Darnell, C., Catchot, A., Musser, F., Cook, D., Dodds, D., Gore, J., & Morsello, S. (2015). Susceptibility of tobacco thrips, Frankliniella fusca, to the neonicotinoid class of insecticides in Mid-South region. In *Proceedings 2015 Beltwide Cotton Conferences, National Cotton Council of America* (pp. 5-7).

Deguine, J. P., & Vaissayre, M. (2007). 23 IPM Case Studies: Cotton. *Aphids as Crop Pests*, 573.

Deguine, J. P., Ferron, P., & Russell, D. (2008). Sustainable pest management for cotton production. A review. *Agronomy for Sustainable Development*, **28**(1), 113-137.

Desneux, N., Decourtye, A., & Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review Entomology*, **52**, 81-106.

Dively, G. P., Venugopal, P. D., Bean, D., Whalen, J., Holmstrom, K., Kuhar, T. P., ... & Hutchison, W. D. (2018). Regional pest suppression associated with widespread Bt maize adoption benefits vegetable growers. *Proceedings of the National Academy of Sciences*, **115**(13), 3320-3325

Dorman, S. J., Gross, A. D., Musser, F. R., Catchot, B. D., Smith, R. H., Reisig, D. D., ... & Taylor, S. V. (2020). Resistance monitoring to four insecticides and mechanisms of resistance in Lygus lineolaris Palisot de Beauvois (Hemiptera: Miridae) populations of southeastern USA cotton. *Pest Management Science*, **76**(12), 3935-3944.

Douglas, M. R., & Tooker, J. F. (2015). Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. *Environmental Science & Technology*, **49**(8), 5088-5097.

Dowd-Uribe, B. (2014). Engineering yields and inequality? How institutions and agro-ecology shape *Bt* cotton outcomes in Burkina Faso. *Geoforum*, **53**, 161-171.

Elbert, A., Haas, M., Springer, B., Thielert, W., & Nauen, R. (2008). Applied aspects of neonicotinoid uses in crop protection. *Pest Management Science: formerly Pesticide Science*, **64**(11), 1099-1105.

Ellsworth, P. C., & Barkley, V. (2005). Transitioning Lygus chemical controls to more selective options for Arizona Cotton. *Cotton: A College of Agriculture and Life Science Report*, **az1366**

Elkassabany, N. M. (1994). *Biology of the insidious flower bug Orius insidiosus (Say)(Heteroptera: Anthocodoridae)* (Doctoral dissertation, University of Arkansas).

Fernandez-Cornejo, J., & Caswell, M. F. (2006). The first decade of genetically engineered crops in the United States. *USDA-ERS Economic Information Bulletin*, **11**.

Fernandez-Cornejo, J., Wechsler, S., Livingston, M., & Mitchell, L. (2014). Genetically engineered crops in the United States. *USDA-ERS Economic Research Report*, (**162**).

Graham, S. H., & Stewart, S. D. (2018). Field study investigating Cry51Aa2. 834_16 in cotton for control of thrips (Thysanoptera: Thripidae) and tarnished plant bugs (Hemiptera: Miridae). *Journal of Economic Entomology*, **111**(6), 2717-2726.

Gross, K., & Rosenheim, J. A. (2011). Quantifying secondary pest outbreaks in cotton and their monetary cost with causal-inference statistics. *Ecological Applications*, **21**(7), 2770-2780.

Gowda, A., Rydel, T. J., Wollacott, A. M., Brown, R. S., Akbar, W., Clark, T. L., Flasinski, S., Nageotte, J.R., Read, A.C., Shi, X., Werner, B.J., Pleau, M.J., & Baum, J. A. (2016). A transgenic approach for controlling Lygus in cotton. *Nature Communications*, **7**(1), 1-7.

Gutierrez, A. P. (2005). Tritrophic effects in *Bt* cotton. *Bulletin of Science, Technology & Society*, **25**(4), 354-360.

Head, G., Moar, W., Eubanks, M., Freeman, B., Ruberson, J., Hagerty, A., & Turnipseed, S. (2005). A multiyear, large-scale comparison of arthropod populations on commercially managed *Bt* and non-*Bt* cotton fields. *Environmental Entomology*, **34**(5), 1257-1266.

Herbert, A., Chappell, T., Huseth, A., Kennedy, G., Reisig, D., Greene, J., Reay-Jones, F., Roberts, P., Toews, M., Jacobson, A., Smith, R. (2021). Managing Thrips in Cotton: Research in the Southeast Region. *Cotton Cultivated*. https://cottoncultivated.cottoninc.com/wp-content/uploads/2016/04/Thrips-Management-in-SE.pdf

Höfte, H., & Whiteley, H. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Reviews*, **53**(2), 242-255.

Huang, J., Hu, R., Pray, C.E., Qiao, F., & Rozelle, S. (2003). Biotechnology as an alternative to chemical pesticides: a case study of *Bt* cotton in China. *Agricultural Economics*, **29**, 55–67.

Hutchison, W. D., Burkness, E. C., Mitchell, P. D., Moon, R. D., Leslie, T. W., Fleischer, S. J., ... & Raun, E. S. (2010). Areawide suppression of European corn borer with *Bt* maize reaps savings to non-*Bt* maize growers. *Science*, **330**(6001), 222-225.

Huseth, A. S., Chappell, T. M., Langdon, K., Morsello, S. C., Martin, S., Greene, J. K., Herbert, A., Jacobson, A.L., Reay-Jones, F., Reed, T., Reisig, D.D., Roberts, P.M., Smith, R., & Kennedy, G. G. (2016). *Frankliniella fusca* resistance to neonicotinoid insecticides: an emerging challenge for cotton pest management in the eastern United States. *Pest Management Science*, **72**(10), 1934-1945.

Huseth, A. S., Chappell, T. M., Chitturi, A., Jacobson, A. L., & Kennedy, G. G. (2018). Insecticide resistance signals negative consequences of widespread neonicotinoid use on multiple field crops in the US cotton belt. *Environmental Science & Technology*, **52**(4), 2314-2322.

Huseth, A. S., D′ Ambrosio, D. A., Yorke, B. T., Head, G. P., & Kennedy, G. G. (2020). Novel mechanism of thrips suppression by Cry51Aa2. 834_16 *Bt* toxin expressed in cotton. *Pest Management Science*, **76**(4), 1492-1499.

Jurat-Fuentes, J. L., Heckel, D. G., & Ferré, J. (2021). Mechanisms of resistance to insecticidal proteins from Bacillus thuringiensis. *Annual Review of Entomology*, **66**, 121-140.

Kennedy, G. G. (2008). Integration of insect-resistant genetically modified crops within IPM programs. *Integration of Insect-Resistant genetically modified crops within IPM programs*, 1-26.

Kim, Y. J., Kloos, S., Romeis, J., & Meissle, M. (2021). Effects of mCry51Aa2-producing cotton on the non-target spider mite Tetranychus urticae and the predatory bug Orius majusculus. *Journal of Pest Science*, **94**(2), 351-362.

Krishna, V. V., & Qaim, M. (2012). *Bt* cotton and sustainability of pesticide reductions in India. *Agricultural Systems*, **107**, 47-55.

Leigh, T. F. (1963). The influence of two systemic organophosphates on growth, fruiting, and yield of cotton in California. *Journal of Economic Entomology*, **56**(4), 517-522.

Liu, Y. B., Tabashnik, B. E., Dennehy, T. J., Patin, A. L., & Bartlett, A. C. (1999). Development time and resistance to *Bt* crops. *Nature*, **400**(6744), 519-519.

Lu, Y., & Wu, K. (2011). Mirid bugs in China: pest status and management strategies. *Outlooks on Pest Management*, **22**(6), 248-252.

Lundgren, J. G., & Wiedenmann, R. N. (2005). Tritrophic interactions among *Bt* (Cry3Bb1) corn, aphid prey, and the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Environmental Entomology*, **34**(6), 1621-1625.

Luttrell, R. G., Fitt, G. P., Ramalho, F. S., & Sugonyaev, E. S. (1994). Cotton pest management: Part 1. A worldwide perspective. *Annual Review of Entomology*, **39**(1), 517-526.

Mall T., Gupta M., Dhadialla T.S., & Rodrigo S. (2019). Overview of Biotechnology-Derived Herbicide Tolerance and Insect Resistance Traits in Plant Agriculture. In: Kumar S., Barone P., Smith M. (eds) Transgenic Plants. *Methods in Molecular Biology*, **1864**. Humana Press, New York, NY.

Maienfisch, P., Angst, M., Brandl, F., Fischer, W., Hofer, D., Kayser, H., Werner, K., Rindlisbacher, A., Senn, R., Steinemann, A., & Widmer, H. (2001). Chemistry and biology of thiamethoxam: a second generation neonicotinoid. *Pest Management Science*, **57**(10), 906-913.

McNaughton, S. J. (1983). Compensatory plant growth as a response to herbivory. *Oikos*, 329-336.

Müller, C. (2018). Impacts of sublethal insecticide exposure on insects—Facts and knowledge gaps. *Basic and Applied Ecology*, **30**, 1-10.

Naranjo, S. E. (2005). Long-term assessment of the effects of transgenic *Bt* cotton on the abundance of nontarget arthropod natural enemies. *Environmental Entomology*, **34**(5), 1193-110.

Orford, S., Delaney, S., & Timmis, J. (2007). The genetic modification of cotton. *Cotton: Science and Technology*, **1**, 103-129.

Palumbo, J. C., Horowitz, A. R., & Prabhaker, N. (2001). Insecticidal control and resistance management for *Bemisia tabaci*. *Crop Protection*, **20**(9), 739-765.

Pesticide Control Act, 7 U.S. Code § 136 (1972). [Online] https://www.govinfo.gov/content/pkg/STATUTE-86/pdf/STATUTE-86-Pg973.pdf [Accessed April 13th, 2022]

Qiao, F. (2015). Fifteen years of *Bt* cotton in China: the economic impact and its dynamics. *World Development*, **70**, 177-185.

Quisenberry, J. E., & Rummel, D. R. (1979). Natural resistance to thrips injury in cotton as measured by differential leaf area reduction. *Crop Science*, **19**(6), 879-881.

Race, S. R. (1965). Predicting thrips populations on seedling cotton. *Journal of Economic Entomology*, **58**(5), 1013-1014.

Reay-Jones, F. P., Greene, J. K., Herbert, D. A., Jacobson, A. L., Kennedy, G. G., Reisig, D. D., & Roberts, P. M. (2017). Within-plant distribution and dynamics of thrips species (Thysanoptera: Thripidae) in cotton. *Journal of Economic Entomology*, **110**(4), 1563-1575.

Reisig, D.D., Huseth, A.S., Bacheler, J.S., Aghaee, M.A., Braswell, L., Burrack, H.J., Flanders, K., Greene, J.K., Herbert, D.A., Jacobson, A., Paula-Moraes, S.V., Roberts, P., & Taylor, S.V. (2018a). Long-term empirical and observational evidence of practical *Helicoverpa zea* resistance to cotton with pyramided *Bt* toxins. *Journal of Economic Entomology*, **111**(4), 1824-1833.

Reisig, D. D., & Kurtz, R. (2018b). *Bt* resistance implications for *Helicoverpa zea* (Lepidoptera: Noctuidae) insecticide resistance management in the United States. *Environmental Entomology*, **47**(6), 1357-1364.

Ruberson, J., Nemoto, H., & Hirose, Y. (1998). Pesticides and conservation of natural enemies in pest management. In *Conservation Biological Control* (pp. 207-220). Academic Press.

Rummel, D. R., & Quisenberry, J. E. (1979). Influence of thrips injury on leaf development and yield of various cotton genotypes. *Journal of Economic Entomology*, **72**(5), 706-709.

Sadras, V. O., & Wilson, L. J. (1998). Recovery of cotton crops after early season damage by thrips (Thysanoptera). *Crop Science*, **38**(2), 399-409.

Sánchez-Bayo, F., & Tennekes, H. A. (2017). Assessment of ecological risks of agrochemicals requires a new framework. *Environmental Risk Assesment. Remediat*ed, **1**(3), 20-28.

Schuler, T. H., Potting, R. P., Denholm, I., & Poppy, G. M. (1999). Parasitoid behaviour and *Bt* plants. *Nature*, **400**(6747), 825-826.

Schuler, T. H., Potting, R. P., Denholm, I., Clark, S. J., Clark, A. J., Stewart, C. N., & Poppy, G. M. (2003). Tritrophic choice experiments with *Bt* plants, the diamondback moth (*Plutella xylostella*) and the parasitoid *Cotesia plutellae*. *Transgenic Research*, **12**(3), 351-361.

Shahid, M. R., Shakeel, M., Farooq, M., Ahmad, S., & Mahmood, A. (2019). Tritrophic Association between *Bt* Cotton, Arthropod Pest and Natural Enemies. *Advances in Cotton Research*.

Shelton, A. M., Zhao, J. Z., & Roush, R. T. (2002). Economic, ecological, food safety, and social consequences of the deployment of *Bt* transgenic plants. *Annual review of entomology*, **47**(1), 845-881.

Shelton, A. M., Romeis, J., Naranjo, S. E., Tian, J., & Hellmich, R. L. (2016). Use of *Bt*-resistant caterpillars to assess the effect of Cry proteins on beneficial natural enemies. *IOBC-WPRS Bulletin*, **114**, 51-55.

Sivasupramaniam, S., Moar, W.J., Ruschke, L.G., Osborn, J.A., Jiang, C., Sebaugh, J.L., Brown, G.R., Shappley, Z.W., & Greenplate, J. T. (2014). Toxicity and characterization of cotton expressing *Bacillus thuringiensis* Cry1Ac and Cry2Ab2 proteins for control of lepidopteran pests. *Journal of Economic Entomology*, **101**(2), 546-554.

Siviter, H., & Muth, F. (2020). Do novel insecticides pose a threat to beneficial insects?. *Proceedings of the Royal Society B*, **287**(1935), 20201265.

Stark, J. D., & Banks, J. E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology*, **48**(1), 505-519.

Tabashnik, B. E., & Carrière, Y. (2017). Surge in insect resistance to transgenic crops and prospects for sustainability. *Nature Biotechnology*, **35**(10), 926-935.

Tian, J. C., Yao, J., Long, L. P., Romeis, J., & Shelton, A. M. (2015). *Bt* crops benefit natural enemies to control non-target pests. *Scientific Reports*, **5**(1), 1-9.

USDA (2021). United States Department of Agriculture. Agricultural Outlook Forum. [Online] https://www.usda.gov/sites/default/files/documents/cotton-outlook.pdf [Accessed April 13th, 2022].

USDA-ARS (2021). United States Department of Agriculture Agriculture Research Service. 2021 Annual Report. [Online]. https://www.ars.usda.gov/research/project/?accnNo=438164&fy=2021 [Accessed February 10th, 2022].

USDA-ERS (2020). United States Economic Research Service**.** Cotton Sector at a Glance. [Online]. https://www.ers.usda.gov/topics/crops/cotton-wool/cotton-sector-at-a-glance/ [Accessed March 2nd, 2022].

USDA-FAS (2022). United States Foreign Agricultural Service. Cotton: World Markets and Trade. [Online]. https://apps.fas.usda.gov/psdonline/circulars/cotton.pdf [Accessed February 9th, 2022].

USDA-NASS. (2017). USDA National Agricultural Statistics Service. [Online]. https:// quickstats.nass.usda.gov/results/D8BD32CC-B671-338A-9687- DD59CEF09CD3 [Accessed April 13th, 2022].

Vaeck, M., Reynaerts, A., Höfte, H., Jansens, S., De Beuckeleer, M., Dean, C., Zabeau, M., Van Montagu, M., & Leemans, J. (1987). Transgenic plants protected from insect attack. *Nature*, **328**(6125), 33-37.

Watts, J. (1937). Reduction of cotton yield by thrips. *Journal Economic Entomology*, **30**:860-863

Wene, G. P., Carruth, L. A., Telford, A. D., & Hopkins, L. (1965). *Arizona Cotton Insects: Descriptions and Habits*. College of Agriculture, University of Arizona (Tucson, AZ).

Wilson, L. J., Bauer, L. R., & Walter, G. H. (1996). 'Phytophagous' thrips are facultative predators of twospotted spider mites (Acari: Tetranychidae) on cotton in Australia. *Bulletin of Entomological Research*, **86**(3), 297-305.

Wilson, L., Downes, S., Khan, M., Whitehouse, M., Baker, G., Grundy, P., & Maas, S. (2013). IPM in the transgenic era: a review of the challenges from emerging pests in Australian cotton systems. *Crop and Pasture Science*, **64**(8), 737-749.

Zeilinger, A. R., Olson, D. M., & Andow, D. A. (2016). Competitive release and outbreaks of non-target pests associated with transgenic *Bt* cotton. *Ecological Applications*, **26**(4), 1047-1054.

Chapter 2

Baseline Susceptibility of *Frankliniella fusca* and *Frankliniella occidentalis* (Thysanoptera: Thripidae) to Cry51Aa2.834_16 *Bt* toxin expressed in cotton

Abstract

In response to gaps in cotton (*Gossyipum hirsutum* L.) pest control, Bayer Crop Science developed the first genetically engineered (GE) trait for targeting piercing-sucking thysanopteran and hemipteran pests. The trait will be marketed as ThryvOn™ and will provide pest control from early season thrips damage. Given the novelty of the trait, this study aims to provide a baseline and framework for evaluating susceptibility of tobacco thrips (*Frankliniella fusca* Hinds), and western flower thrips (*Frankliniella occidentalis* Pergande). To do this, thrips populations were collected from various cotton growing regions primarily in the Southeastern United States and reared to evenly aged F_1 adult cohorts. The thrips were assayed in a no-choice experiment to measure mortality and oviposition of individual adult females who were exposed to either ThryvOn or near-isoline (non-*Bt*) plant tissue over 6 days. Limited mortality impacts were observed between ThryvOn and non-*Bt* cotton treatments, however, our results show significant oviposition deterrence in all populations tested across both thrips species. These results improve confidence in efficacy of pest control through population reduction as a direct result of decreased oviposition. Additionally, the results provide an important baseline for susceptibility data for ongoing resistance monitoring efforts for this new technology.

Keywords: tobacco thrips, western flower thrips, *Gossypium hirsutum,* MON 88702

Introduction

Genetically engineered insecticidal toxins have been the foundation of Integrated Pest Management in upland cotton (*Gossypium hirsutum* L.) for more than two decades (Fitt and Wilson, 2000; Naranjo, 2011). In 2020, an estimated 4.3 million hectares of U.S. cotton (~88% of domestic production) were planted with varieties that express multiple insecticidal *Bacillus thuringiensis* (*Bt*) toxins targeting economically important lepidopteran pests of the crop (i.e., *Choridea virescens* Fabricius, *Helicoverpa zea* Boddie, *Pectinophora gossypiella* Saunders) (USDA-ERS 2020). Over time, this widespread transition to *Bt* cotton varieties has improved the sustainability of the production system through incremental reductions in pesticide inputs targeting lepidopteran pests (Tabashnik *et al*., 2013). Despite the significant improvement in management of these lepidopteran herbivores, several key pests of cotton are not controlled by commercially available *Bt* toxins.

In response to gaps in the cotton pest spectrum, Bayer Crop Science developed the first genetically engineered (GE) trait for control of piercing-sucking pests (Bachman *et al*., 2017). The new trait expresses Cry51Aa2.834_16 and will be marketed under the trade name ThryvOn™ as stacked trait in combination with lepidopteran-active toxins (e.g., Bollgard 3: Cry1Ac, Cry2Ab, and Vip3a; Bayer 2022). The Cry51Aa2.834_16 protein (hereafter referred to as ThryvOn) was modified from Cry51Aa2 for increased activity against hemipteran and thysanopteran pests (Gowda *et al*., 2016; Jerga *et al*., 2016). In the U.S. Cotton Belt, ThryvOn will target tarnished plant bug (*Lygus lineolaris* Palisot de Beauvois), western tarnished plant bug (*Lygus hesperus* Knight), tobacco thrips (*Frankliniella fusca* Hinds), and western flower thrips (*Frankliniella occidentalis* Pergrande) (Akbar *et al*., 2019). Currently, several of these pests are managed with multiple insecticide applications and, as a result, transition to this GE

trait is anticipated to have considerable benefits for sustainable pest management in cotton (Akbar *et al*., 2019). Although the ThryvOn trait will limit economic injury from plant bugs, control of early season thrips populations will be a major benefit for farmers, particularly in the eastern part of the U.S. Cotton Belt (Huseth *et al*. 2020b).

Adult thrips infest and reproduce on susceptible cotton from emergence through the first four to five weeks following plant emergence (Cook *et al*., 2011). Direct feeding by adults and larval life stages can cause direct damage to plant epidermal cells, which may result in a wrinkled or depressed leaf appearance, malformation, loss of apical dominance, reduction in root growth, and plant mortality in severe infestations (Cook *et al*., 2011). Intense early season thrips seedling injury can cause a long-term reduction in plant health that is thought to reduce drought tolerance and cotton lint yield at the end of the season (Cook *et al*., 2003; Cook, 2011). Many thrips species have been documented on seedling cotton in the United States, including tobacco thrips (*Frankliniella fusca* Hinds), flower thrips (*F. tritici* Fitch), western flower thrips (*F. occidentalis* Pergande), onion thrips (*Thrips tabaci* Lindeman), and soybean thrips (*Neohydatothrips variabilis* Beach) (Cook *et al*., 2011; Reay-Jones *et al*., 2017; Wang *et al*., 2018). However, the most economically important seedling pest of cotton in the eastern U.S. is *F. fusca* (Cook *et al*., 2011). A major barrier to developing innovative IPM strategies for thrips is the variability of thrips infestation intensity each year. The severity of *F. fusca* infestations are related to both the timing of adult dispersal from alternate hosts as well as the relative susceptibility of the cotton crop, which is influenced by the growth rate of cotton and local weather patterns (Lewis, 1965; Belder *et al*., 2001; Kerns *et al*., 2002; Chappell *et al*., 2020). Currently there are thrips prediction tools available that use several inputs such as planting date

and weather patterns to ascertain whether control is necessary for thrips given seasonal densities (Chappell *et al.*, 2020).

To address spatially and temporally variable *F. fusca* infestations, farmers have adopted prophylactic insecticide treatments at broad scales (Cook, 2011; Reitz *et al*., 2020). These treatments can include neonicotinoid seed treatments (imidacloprid and thiamethoxam), at-plant granular, liquid or in-furrow insecticides (aldicarb, imidacloprid), or foliar-applied organophosphates (acephate) (Cook, 2011; Herbert *et al.*, 2021). For the past several decades, neonicotinoid seed treatments have been the cornerstone for *F. fusca* control in U.S. cotton (Douglas and Tooker, 2015; Hurley and Mitchell, 2020). As a result of widespread and repeated seed treatment use, neonicotinoid-resistant *F. fusca* populations are now prevalent throughout the Cotton Belt (Huseth *et al*., 2016 & 2018; Darnell-Crumpton *et al*., 2018; Chappell *et al*., 2019). Farmers have responded to this problem by including additional in-furrow and foliar insecticide sprays, a transition that increases the insecticide footprint of cotton between 6.1 and 15.1 fold relative to the seed treatment alone (Huseth *et al*., 2016).

To address *F. fusca* neonicotinoid resistance, alternative management strategies will be crucial to alleviate reliance on insecticides that not only limit crop damage, but also increase variable economic costs and environmental externalities of cotton production (Huseth *et al*. 2018). As part of the *F. fusca* Insecticide Resistance Management (IRM) strategy, cotton varieties expressing the ThryvOn toxin will provide an important new tool to manage resistant populations while simultaneously reducing the need for supplemental foliar insecticides. In an initial field study of the novel *Bt* toxin, Graham and Stewart (2018) demonstrated that ThryvOn cotton reduced *F. fusca* counts by 71.3% relative to the non-*Bt* isoline (Graham and Stewart, 2018). Subsequent laboratory and greenhouse studies focused on ThryvOn detected similar

population suppression effects under both choice and no-choice experimental designs (Graham *et al*., 2019; Huseth *et al*., 2020a; D'Ambrosio *et al*., 2020a; D'Ambrosio *et al*., 2020b). In series of targeted behavioral studies, researchers documented a direct reduction in female *F. fusca* oviposition events when exposed ThryvOn cotton tissue under no-choice conditions over an 18 day period (Huseth *et al*. 2020a). However, the ThryvOn toxin did not have acute or chronic lethal effects on either adult or immature *F. fusca* (Huseth *et al*., 2020a), a result that complicates IRM recommendations based on the putative mechanism underlying ThryvOn control. To further refine understanding of the effect of this toxin in more realistic exposure scenarios, researchers conducted a series of choice experiments to emulate field conditions where female thrips could disperse to alternate hosts after toxin exposure (Huseth *et al*., 2020a & b). Results of choice experiments confirmed that the suppression effect was related to female *F. fusca* reproductive behavior, suggesting that the primary mechanism for *F. fusca* involved an interaction between host plant suitability and reproductive decisions (Huseth *et al*., 2020b). A subsequent experiment using electropenetrography (EPG) documented that female *F. fusca* probed and ingested less on ThryvOn cotton relative to non-*Bt* isoline implying this would be the first genetically modified toxin that works in part through antixenosis (D'Ambrosio *et al*., 2020a).

Together, these laboratory studies provide evidence that non-lethal effects drive *F. fusca* population suppression and, as a result, regulatory interpretation of the appropriate IRM tactics will be different than principles used to estimate durability for previous insect-active *Bt* toxins targeting lepidopteran pests. One crucial knowledge gap that limits future ThryvOn IRM plans is a firm understanding of baseline susceptibility among wild *F. fusca* populations and a related cotton pest, *F. occidentalis*. In this study, we exposed field-collected *F. fusca* and *F. occidentalis* populations collected across the U.S. Cotton belt to ThryvOn and a non-*Bt* isoline under no-

choice laboratory conditions. Because previous studies documented limited lethal effects of ThryvOn in *F. fusca*, we hypothesized that wild adults would not have significantly different mortality rates from laboratory reared colonies. We also hypothesized that the oviposition suppression would be common among wild populations based on published field experiments documenting significant reductions of larval counts and seedling damage estimates (Akbar *et al*., 2019, Graham and Stewart, 2018). To do this, we combined laboratory ThryvOn mortality assays with a leaf-clearing assay designed to estimate oviposition events on an individual female basis. Outcomes of this study provide important insight into the variation in ThryvOn's oviposition suppression and non-lethal effects among wild thrips populations. These baseline efficacy studies also provide an important foundation for resistance management plans targeting thysanopteran-active *Bt* toxins in cotton.

Materials and Methods

Thrips populations and experimental cotton seedlings

Thrips populations were collected in the spring of 2021 from either untreated cotton seedlings, small grains, or tomato (Table 2.1). All sample locations were in cotton producing regions with the exception of one *F. occidentalis* population collected from a tomato producing area in Rowan County, NC. In each cotton plot, newly emerged untreated seedlings were randomly harvested into buckets that included paper towels separating seedling layers to limit condensation (Huseth *et al*., 2016). Populations from small grains were collected using a thrips-proof mesh sweep net at grain maturity. In tomato, *F. occidentalis* were collected from blossoms by vigorously shaking plants onto white paper and aspirating adults. Collections from outside North Carolina were shipped to North Carolina State University in sealed 18.9 L buckets that were layered with host material to provide a food source during shipment. All sample buckets

were equipped with freezer packs adhered to the outside to ensure collections did not overheat. Upon arrival, adult female thrips were aspirated from plant material and transferred to fresh cabbage contained into 4.9 L plastic container cages (0.35 L; VP-1257064, PFS Sales Company, Raleigh, NC, USA) equipped with air vents made of thrips-proof screen (81 × 81 mesh; Bioquip Products, Inc., Rancho Dominguez, CA). Field-collected female adults were maintained in the laboratory on insecticide-free cabbage to rear a cohort of larvae. Three days after pupation, F1 adult female thrips were entered into no-choice assays. The F1 cohort was used in all subsequent experimental assays. Laboratory-reared *F. fusca* and *F. occidentalis* insecticide-susceptible colonies were included as susceptible controls in each assay.

To generate plant material for bioassays, ThryvOn and a non-*Bt* near-isoline variety (DeltaPine 1646) cotton seedlings were grown in the Method Road Greenhouse located on NC State University Campus. Seedlings were grown in 16 cell plastic greenhouse flats filled with a sterile soil mix (two parts loam, two peat, one sand), and contained in thrips-proof cages to limit unintentional thrips infestations in the greenhouse (BugDorm-2120F, MegaView Science Co., Ltd., Taiwan). Seedlings were grown for 7-10 days until cotyledons fully expanded before they were used for bioassays. All seeds used in this study were provided by Bayer CropScience, Chesterfield, MO.

Adult mortality

An 11.3 mm internal diameter cork borer was used to cut cotton tissue from cotyledons, avoiding any major leaf venation following the methods of Huseth *et al*., (2020a). Individual leaf disks were placed in a 1.5 mL microcentrifuge tube (#05-408-130, Fisher Scientific International Inc., Pittsburgh, PA, USA) and a single three-day old adult female thrips was aspirated into the tube. A total of 100 female thrips were included in both the ThryvOn and non-*Bt* treatments for

each population. After aspirating was completed, insects were held in foam tube racks (PolarSafe™ EPS Cryogenic Storage Racks, Argos Technologies, Vernon Hills, IL) in a temperature-controlled growth chamber at 27°C (12:12 light to dark photoperiod). Mortality was evaluated every 24 hours for 6 consecutive days using stereomicroscope. Insects were classified as either alive, dead, or moribund. Moribund insects were classified as those who did not change position after disturbance with a soft bristled, fine paint brush. Leaf disks from dead insects were immediately stored in a 3°C refrigerator until leaf staining could be completed. Due to differences in egg eclosion time between the thrips species, leaf disks were changed every three days for *F. fusca,* and every two days for *F. occidentalis* (Huseth *et al*., 2020a). At the conclusion of the 6-day exposure window, all insects were terminated prior to leaf clearing to estimate oviposition.

Oviposition rate over time

To understand oviposition deterrence, we evaluated average oviposition rate per day on each treatment. Leaf disks were cleared and stained following methods published in previous literature (Simonet and Pienkowski 1977; Nuessly *et al*., 1995; Riley *et al*., 2007). After completion of staining, the leaf disks were assessed for *F. fusca* and *F. occidentalis* eggs under the aid of a backlit stereomicroscope. Through the entirety of the experiment, individual thrips were tracked with the corresponding leaf disk to ensure coordinated data analysis of thrips mortality and oviposition rate could be conducted by individual. Depending on the individual mortality outcomes and developmental differences, the *F. occidentalis* could have a maximum of three leaf disks whereas the *F. fusca* could have a maximum of two leaf disks for fecundity counts.

Statistical Analysis

All statistical analyses were conducted using RStudio statistical software (Version 1.3.1093 R Core Team, 2020. R packages, *emmeans*, *car*, *multcomp*, *dplyr*, were used to analyze and visualize data (Lenth, 2020; Fox, 2021; Hothorn, 2021; Wickham *et al.*, 2019). Each thrips species was evaluated for mortality response to ThryvOn and non-*Bt* treatments independently. The mortality after a six-day exposure was modeled using a generalized linear model (GLM) fitted with a binomial distribution (link: logit) testing main effects of population, plant treatment, and their interaction. Post-hoc contrasts among populations were estimated using Tukey's HSD with the *emmeans* package (Lenth, 2020).

To relate population and plant treatment effects to oviposition, we first calculated the average number of eggs per day for each individual female thrips. Because some individuals died during the course of the exposure window, this calculation accounted for differential survival rates. Oviposition rates were analyzed and conducted separately for each species with linear models fitted with a gaussian distribution. Models included main effects of population, plant treatment, and their interaction. Type III ANOVAs were used for evaluating significance. All pairwise comparisons were made using Tukey's HSD with the *emmeans* package (Lenth, 2020).

Results

In the spring of 2021, seven *F. fusca* populations were collected in cotton producing areas located in Louisiana, Mississippi, North Carolina, and Virginia (Table 2.1). *Frankliniella occidentalis* populations were collected in Arizona and North Carolina (Table 2.1). Treatment variety (ThryvOn or non-*Bt*) did not significantly affect the survival of *F. fusca* (Table 2.2). The *F. fusca* populations had significantly different mortality probabilities in the experiment (Table 2.2). Adult *F. fusca* survival was significantly different among locations over the six-day

exposure period (Figure 2.1). However, the interaction term between population and treatment was not significant.

The generalized linear model for the *F. occidentalis* survival assay detected a significant interaction of treatment and population was significant (Table 2.2). The post-hoc analysis found a significant increase in the probability of mortality with the lab colony on the ThryvOn cotton tissue; however, the wild-caught populations did not have equivalent levels of mortality on the ThryvOn tissues (Figure 2.2).

In this study, *F. fusca* oviposition was highly variable among populations, with the population from NC-4 displaying a much lower fecundity than other field-caught populations in both treatments (Table 2.3). Model results showed a significant interaction between treatment and population (Table 2.3). A consistent reduction in the number of eggs was observed on ThryvOn tissue relative to non-*Bt* for all *F. fusca* populations (Figure 2.3). On average, thrips on the ThryvOn *Bt* plant tissue had a 1.2-fold reduction in the average number of eggs when compared to non-*Bt* plant tissue.

All three *F. occidentalis* populations, including the laboratory susceptible population, had similar oviposition results to *F. fusca*. The model detected a significant interaction between treatment and populations (Table 2.3). Every *F. occidentalis* population had a significant decline in fecundity when exposed to the ThryvOn toxin (Figure 2.4). The laboratory population had the lowest fecundity when exposed to the ThryvOn cotton tissue, and on average, a 2-fold difference in the average number of *F. occidentalis* eggs on ThryvOn when compared to the non-*Bt* cotton tissue (Table 1.3).

Discussion

In this study we assessed mortality and oviposition rates in field-collected thrips populations from the southeast, mid-south, and western cotton producing regions. The study addresses key knowledge gaps about the performance of the ThryvOn toxin against field-collected thrips under controlled laboratory conditions. Here, we generated important baseline mortality and oviposition suppression data for field-collected *F. fusca* and *F. occidentalis* that will be informative for regulatory and industry stakeholders who are concerned with uncertainty about ThryvOn efficacy among populations and the unknown differences in performance among major cotton producing regions. As an important note, our study also controls for the behavioral avoidance by using a no-choice assay to distinguish the direct impact of toxin exposure on mortality and fecundity; the use of a no-choice assay implies that our results may be more conservative compared to field oviposition rates given the preference of thrips to oviposit on non-*Bt* cotton tissue addressed in previous studies (Graham and Stewart 2018; Graham *et al*., 2019; Huseth *et al*., 2019). The results add to the growing body of evidence that ThryvOn is not an acutely toxic to *Frankliniella* spp. adults, however, the toxin does appear to affect the fitness of female thrips through oviposition suppression (Graham *et al*., 2019; Huseth *et al*., 2019; D'Ambrosio *et al*., 2020; Huseth *et al*., 2020).

In our six-day mortality study, the ThryvOn trait had variable impacts on *F. occidentalis* mortality across differing populations, but the toxin did not significantly impact mortality in wild or laboratory *F. fusca* populations. Overall, *F. occidentalis* were more sensitive to ThryvOn which is consistent with previous findings (D'Ambrosio *et al*., 2020b; Huseth *et al*., 2020). The lack of mortality from acute exposure, across multiple thrips species, is not a new discovery with

this toxin. However, our study does confirm that limited direct mortality occurs in field-collected populations in addition to the lab-populations (Graham *et al*., 2019; Huseth *et al*., 2020).

In our study we found that sub-lethal effects reduce the number of oviposition events, a reasonable explanation for population suppression in the field (Graham *et al.*, 2019; Huseth *et al.*, 2020). While oviposition suppression occurred, it is of important to note that ThryvOn does not completely deter egg laying; however, previous literature reports for high mortality for *F. occidentalis* larvae, implying that a cohort of eggs laid on ThryvOn would be unlikely to fully develop (Huseth *et al*., 2019). Current insecticidal resistance management approaches suggest a high-dose strategy to prevent resistance that may not be applicable to ThryvOn, consequently there is potential for a quicker evolution of resistance given the survival and selection pressure of implementing a pest management technique that works on multivoltine pests predominantly through sub-lethal tactics (Gould, 1998; Huseth *et al.*, 2020b).

Conclusions and Future Directions

Given the unique activity of this technology, baseline sensitivity towards the trait is important in both the resistance management, and pest management recommendations that coincide with commercialization of ThryvOn. Here we provided a baseline and framework to track susceptibility to the novel ThryvOn technology that may continue to be referenced for resistance management after deployment. Tracking efficacy of this toxin over time will help us gather a better understanding of the durability of a control that works through behavioral avoidance and antixenosis. Additionally, the deployment of ThryvOn will better inform how long-term resistance management programs will need to be tailored for sub-lethal control techniques that do not follow established high-dose recommendations that pertain to other insecticide resistant management programs. Furthermore, the deployment of ThryvOn will add

an additional control technique benefitting resistance management of other technologies by diversifying current thrips pest management (Sparks and Nauen, 2015; Akbar *et al.*, 2019).

The results of our study improve confidence that this toxin will suppress oviposition under field conditions. The adoption of ThryvOn will clearly help to simplify early season thrips management via uniform protection provided by a plant-incorporated protectant (Huseth *et al.*, 2020). Integration of ThryvOn cotton into existing production systems could provide an additional tool which may help reduce the reliance on in-furrow and foliar insecticide applications currently being used for neonicotinoid resistant populations and, as a result, will improve environmental footprint of cotton production at a regional scale (Akbar *et al.*, 2019).

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References

Akbar, W., Gowda, A., Ahrens, J.E., Stelzer, J.W., Brown, R.S., Bollman, S.L., Greenplate, J.T., Gore, J., Catchot, A.L., Lorenz, G., Stewart, S.D., Kerns, D.L., Greene, J.K., Toews, M.D., Herbert, D.A., Reisig, D.D., Sword, G.A., Ellsworth, P.C., Godfrey, L.D. and Clark, T.L. (2019). First transgenic trait for control of plant bugs and thrips in cotton. *Pest. Management Science*, **75,** 867-877.

Bachman, P., Ahmad, A., Ahrens, J., Akbar, W., Baum, J., Brown, S., Clark, T., Fridley, J., Gowda, A., Greenplate, J., Jensen, P., Mueller, G., Odegaard, M., Tan, J., Uffman, J., and Levine, S. (2017). Characterization of the activity spectrum of MON 88702 and the plant-incorporated protectant Cry51Aa2.834_16. PLoS ONE **12**(1), e0169409.

Bayer Crop Science. (2022). Bollgard® 3 ThryvOn™ with XtendFlex® Technology. Available at: https://traits.bayer.com/cotton/Pages/bollgard-3-thryvon.aspx [Accessed: 2 February 2022].

Belder, E., Elderson, J., Van den Brink, W., and Schelling, G. (2001). Effect of woodlots on thrips density in leek fields: a landscape analysis *Agriculture, Ecosystems & Environment* **91**(1-3), 139-145.

Chappell, T.M., Huseth, A.S. and Kennedy, G.G. (2019). Stability of neonicotinoid sensitivity in *Frankliniella fusca* populations found in agroecosystems of the southeastern USA. *Pest Management Science*, **75**, 1539-1545.

Chappell, T.M., Ward, R.V., DePolt, K.T., Roberts, P.M., Greene, J.K. and Kennedy, G.G. (2020). Cotton thrips infestation predictor: a practical tool for predicting tobacco thrips (*Frankliniella fusca*) infestation of cotton seedlings in the south-eastern United States. *Pest Management Science*, **76**, 4018-4028.

Cook, D.R., Allen, C.T., Burris, E., Freeman, B.L., Herzog, G.A., Lentz, G.L., Leonard, B.R., & Reed, J. T. (2003). A survey of thrips (Thysanoptera) species infesting cotton seedlings in Alabama, Arkansas, Georgia, Louisiana, Mississippi, and Tennessee. *Journal of Entomological Science*, **38**(4), 669-681.

Cook, D., Herbert, A., Akin, D., Reed, J. (2011). Biology, crop injury, and management of thrips (Thysanoptera: Thripidae) infesting cotton seedlings in the United States, *Journal of Integrated Pest Management* **2**(2), B1-B9.

Cook, W., and Threet, M. (2020). Cotton insect losses [Online]. Mississippi State University. https://www.biochemistry.msstate.edu/resources/pdf/2020/table28.pdf [Accessed: 4 February 2022].

D'Ambrosio, D.A., Kennedy, G.G. and Huseth, A.S. (2020a). Feeding behavior of *Frankliniella fusca* on seedling cotton expressing Cry51Aa2.834_16 *Bt* toxin. *Pest Management Science*, **76**, 2781-2786.

D'Ambrosio, D. A., Kennedy, G. G., & Huseth, A. S. (2020b). *Frankliniella fusca* and *Frankliniella occidentalis* response to thrips-active Cry51Aa2. 834_16 *Bt* cotton with and without neonicotinoid seed treatment. *Crop Protection*, **129**, 105042.

Darnell-Crumpton, C., Catchot, A., Cook, D., Gore, J., Dodds, D., Morsello, & S., Musser, F. (2018). Neonicotinoid insecticide resistance in tobacco thrips (Thysanoptera: Thripidae) of Mississippi, *Journal of Economic Entomology*, **111**(6), 2824–2830

Douglas, M. R., & Tooker, J. F. (2015). Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. *Environmental science & technology*, **49**(8), 5088-5097.

Fitt, G. P., & Wilson, L. J. (2000). Genetic engineering in IPM: Bt cotton. In *Emerging technologies for integrated pest management: concepts, research, and implementation.* Proceedings of a Conference, Raleigh, North Carolina, USA, 8-10 March, 1999. *American Phytopathological Society (APS Press)*. 108-125.

Fox, J. (2021). Package 'car'. https://cran.r-project.org/web/packages/car/car.pdf

Gould F. (1998). Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology* **43**, 701-726.

Gowda, A., Rydel, T., Wollacott, A., Brown, R., Akbar, W., Clark, T., Flasinski, S., Nageotte, J., Read, A., Shi, X., Werner, B., Pleau, M., & Baum, J. (2016). A transgenic approach for controlling *Lygus* in cotton. *Nature Commun*ications, **7**, 12213.

Graham, S.H., & Stewart, S.D. (2018). Field study investigating Cry51Aa2. 834_16 in cotton for control of thrips (Thysanoptera: Thripidae) and tarnished plant bugs (Hemiptera: Miridae). *Journal of Economic Entomology*, ***111***(6), 2717-2726.

Graham, S.H., Musser, F.M., Jacobson, A.L., Chitturi, A., Catchot, B., & Stewart, S.D., (2019). Behavioral responses of thrips (Thysanoptera: Thripidae) and tarnished plant bug (Hemiptera: Miridae) to a new *Bt* toxin, Cry51Aa2. 834_16 in cotton. *Journal of Economic Entomology*, ***112***(4), 1695-1704.

Herbert, A., Chappell, T., Huseth, A., Kennedy, G., Reisig, D., Greene, J., Reay-Jones, F., Roberts, P., Toews, M., Jacobson, A., Smith, R. (2021). Managing Thrips in Cotton: Research in the Southeast Region. *Cotton Cultivated*.

https://cottoncultivated.cottoninc.com/wp-content/uploads/2016/04/Thrips-Management-in-SE.pdf

Hothorn, T., (2021) Package "multcomp". Simultaneous Inference in General Parametric Models. https://cran.r-project.org/web/packages/multcomp/multcomp.pdf

Hurley, T.M., & Mitchell, P.D. (2020). The value of insect management to US maize, soybean and cotton farmers. *Pest Management Science*, **76**(12), 4159-4172.

Huseth, A.S., Chappell, T.M., Langdon, K., Morsello, S.C., Martin, S., Greene, J.K., Herbert, A., Jacobson, A.L., Reay-Jones, F.P., Reed, T., Reisig, D.D., Roberts, P.M., Smith, R. and Kennedy, G.G. (2016). *Frankliniella fusca* resistance to neonicotinoid insecticides: an emerging challenge for cotton pest management in the eastern United States. *Pest Management Science*, **72,** 1934-1945.

Huseth A., Chappell T., Chitturi A., Jacobson A., and Kennedy G. (2018). Insecticide resistance signals negative consequences of widespread neonicotinoid use on multiple field crops in the US cotton belt. *Environmental Science Technology*, **52**(4), 2314–2322.

Huseth, A., D'Ambrosio, D., & Kennedy, G. (2020a). Understanding the potential impact of continued seed treatment use for resistance management in Cry51Aa2.834_16 *Bt* cotton against *Frankliniella fusca*. *PLoS ONE* **15**(10), e0239910.

Huseth, A. S., D′ Ambrosio, D. A., Yorke, B. T., Head, G. P., & Kennedy, G. G. (2020b). Novel mechanism of thrips suppression by Cry51Aa2. 834_16 Bt toxin expressed in cotton. *Pest Management Science*, *76*(4), 1492-1499.

Jerga, A., Chen, D., Zhang, C., Fu, J., Kouadio, J.L.K., Wang, Y., Duff, S., Howard, J., Rydel, T., Evdokimov, A., Ramaseshadri, P., Evans, A., Bolognesi, R., Park, Y., & Haas, J.A. (2016). Mechanistic insights into the first *Lygus*-active β-pore forming protein. *Archives of Biochemistry and Biophysics*, **600**, 1-11.

Kerns, C., Greene, J., Reay-Jones, F., & Bridges, W. (2002). Effects of planting date on thrips (Thysanoptera: Thripidae) in cotton, *Journal of Economic Entomology* **112**(2), 699–707.

Lenth, R. (2020). emmeans: Estimated marginal means, aka least-squares means. https://cran.r-project.org/package=emmeans

Lewis, T., (1965). The species, aerial density and sexual maturity of Thysanoptera caught in mass flights. *Animals of Applied Biology*, **55**(2), 219-225.

Naranjo, S. E. (2011). Impacts of *Bt* transgenic cotton on integrated pest management. *Journal of Agricultural and Food Chemistry*, **59**(11), 5842-5851.

Nuessly, GS., Nagata RT., Skiles ES,. Christenson JR. and Elliott C. (1995). Techniques for differentially staining *Liriomyza trifolii* (Diptera: Agromyzidae) eggs and stipples within cos lettuce leaves. *Florida Entomology*, **78**, 258–264.

Reay-Jones, F. P., Greene, J. K., Herbert, D. A., Jacobson, A. L., Kennedy, G. G., Reisig, D. D., & Roberts, P. M. (2017). Within-plant distribution and dynamics of thrips species (Thysanoptera: Thripidae) in cotton. *Journal of economic entomology*, **110**(4), 1563-1575.

Reitz, S., Gao, Y., Kirk, W., Hoddle, M., Leiss, K., Funder, J. (2020). Invasion biology, ecology, and management of western flower thrips. *Annual Review of Entomology*, **65**(1), 17-37.

Riley D.G., Chitturi A., and Sparks A.N., (2007). Does natural deposition of pine pollen affect the ovipositional behavior of *Frankliniella occidentalis* and *Frankliniella fusca*? *Entomologia Experimentalis et Applicata*, **124**, 133–141.

Simonet D.E., and Pienkowski R.L., (1977). Sampling and distribution of potato leafhopper eggs in alfalfa stems. *Annals of the Entomological Society of America*, **70**(6), 933–936.

Sparks, T., Nauen, R. (2015). IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, **121**, 122-128.

Tabashnik, B.E., Brévault, T., & Carrière, Y. (2013). Insect resistance to *Bt* crops: lessons from the first billion acres. *Nature Biotechnology*, **31**(6), 510-521.

USDA-ERS 2020. United States Department of Agriculture – Economic Research Service (2020). United States Department of Agriculture Economic Research Service (USDA) Recent Trends in GE adoption. [Online]. https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption.aspx [Accessed 22 December 2021].

Wang, S., Just, D., Pinstrup-Andersen, P., (2008). *Bt*-cotton and secondary pests. *International Journal of Biotechnology*, **10**(2-3), 113-121.

Wang, H., Kennedy, G.G., Reay-Jones, F.P., Reisig, D.D., Toews, M.D., Roberts, P.M., Herbert, D.A., Taylor, S., Jacobson, A.L., & Greene, J.K. (2018). Molecular identification of thrips species infesting cotton in the Southeastern United States. *Journal of economic entomology*, **111**(2), 892-898.

Wickham, H., François, R., Henry, L., and Müller, K., (2018). dplyr: A Grammar of Data Manipulation. R package version 0.7.6. https://CRAN.R-project.org/package=dplyr

TABLES

Table 2.1: Population location collection information. Populations were obtained in the Spring and Summer of 2021.

Species	State	Location	Population Name	Collection Date	Host Plant
F. fusca	North Carolina	Tillery, NC	NC-1	5-3-2021	Grain
		Warsaw, NC	NC-2	5-3-2021	Grain
		Robersonville, NC	NC-3	5-10-2021	Grain
		Scotland Neck, NC	NC-4	6-30-2021	Grain
	Mississippi	Starkeville, MS	MS	4-16-2021	Cotton
	Virginia	Suffolk, VA	VA	6-2-2021	Cotton
	Louisiana	St. Joseph, LA	LA	7-14-2021	Cotton
	Laboratory colony		LabSusc	-	Cabbage
F. occidentalis	Arizona	Maricopa, AZ	AZ	6-1-2021	Cotton
	North Carolina	Woodleaf, NC	NC-5	6-23-2021	Tomato
	Laboratory colony		LabSusc	-	Cabbage

Table 2.2: Generalized linear model results of the mortality across the 6-day exposure window for respective *Frankliniella fusca* and *Frankliniella occidentalis* models.

ANOVA (Type III) Mortality Analysis				
Model	**Variable**	χ^2	**DF**	***P*-value**
Frankliniella fusca	Treatment	0.320	1	0.572
	Population	24.816	7	0.001***
	Treatment × Population	4.457	7	0.726
Frankliniella occidentalis	Treatment	5.037	1	0.025*
	Population	4.651	2	0.098
	Treatment × Population	37.618	2	0.000***
	Asterisks represent statistical significance (*P < 0.05, ***P < 0.001).			

Table 2.3: Generalized linear model results of the average thrips oviposition rate per day by treatment and population for respective *Frankliniella fusca* and *Frankliniella occidentalis* models.

ANOVA (Type III) Oviposition by Treatment Analysis				
Model	**Variable**	χ^2	**DF**	**P-Value**
Frankliniella fusca	Population	484.76	7	< 0.001***
	Treatment	79.21	1	< 0.001***
	Population × Treatment	77.97	7	< 0.001***
Frankliniella occidentalis	Population	104.79	2	< 0.001***
	Treatment	92.35	1	< 0.001***
	Population × Treatment	16.50	2	< 0.001***

Asterisks represent statistical significance (*P < 0.05, ***P < 0.001).

Table 2.4: *Frankliniella fusca* oviposition averages per day across experiment separated by population and treatments.

***F. fusca* Oviposition Averages ±95% Confidence Interval**		
Population	**Treatment**	**Average Egg Count Per Day**
LabSusc	non-*Bt*	8.20 ± 0.75
	ThryvOn	3.22 ± 0.34
NC-1	non-*Bt*	7.05 ± 0.51
	ThryvOn	3.90 ± 0.30
NC-2	non-*Bt*	9.22 ± 0.42
	ThryvOn	4.33 ± 0.27
NC-3	non-*Bt*	8.59 ± 0.66
	ThryvOn	4.60 ± 0.40
NC-4	non-*Bt*	2.42 ± 0.78
	ThryvOn	0.94 ± 0.35
VA	non-*Bt*	6.94 ± 0.74
	ThryvOn	1.75 ± 0.29
MS	non-*Bt*	7.59 ± 0.82
	ThryvOn	4.26 ± 0.69
LA	non-*Bt*	8.12 ± 0.72
	ThryvOn	3.97 ± 0.49

Table 2.5: *Frankliniella occidentalis* oviposition averages per day across experiment separated by population and treatments.

***F. occidentalis* Oviposition Averages ±95% Confidence Interval**		
Population	**Treatment**	**Average Egg Count Per Day**
LabSusc	non-*Bt*	2.40 ± 0.27
	ThryvOn	0.26 ± 0.05
NC-5	non-Bt	2.94 ± 0.31
	ThryvOn	1.09 ± 0.11
AZ	non-Bt	4.35 ± 0.42
	ThryvOn	1.43 ± 0.29

FIGURES

Figure 2.1: Percent mortality of *Frankliniella fusca* by population after the 6-day exposure averaged across both treatments. Given the significant fixed effect of population in the (GLM) mortality analysis (P=0.001). Significance shown by letters is from a Tukey HSD on the estimated marginal means.

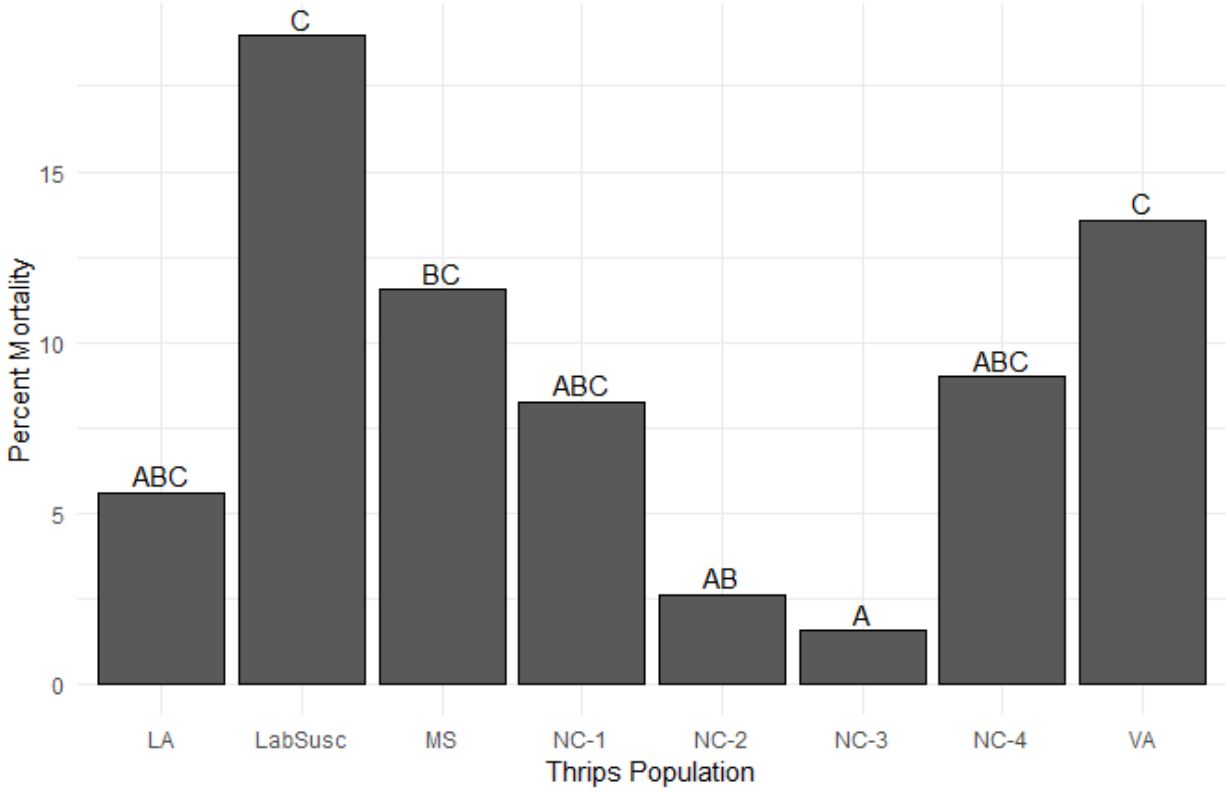


Figure 2.2: Percent mortality of *Frankliniella occidentalis* by population after the 6-day exposure by treatment. Given the significant fixed effect of population × treatment in the (GLM) mortality analysis (P<0.001), Significance shown by letters is from a Tukey HSD on the estimated marginal means.

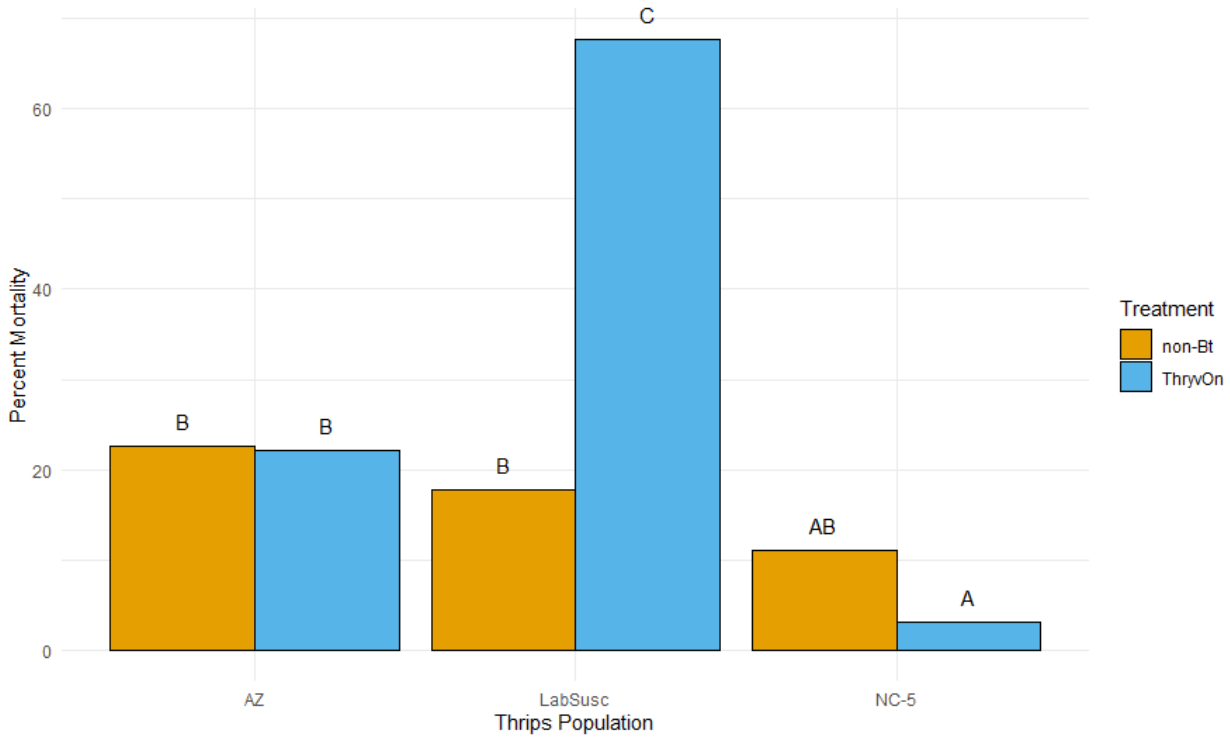


Figure 2.3: Average number of eggs per *F. fusca* female per day separated by location and treatment, with standard error. A Tukey's HSD on the estimated marginal means of the interaction between treatment and location denotes significance by letters.

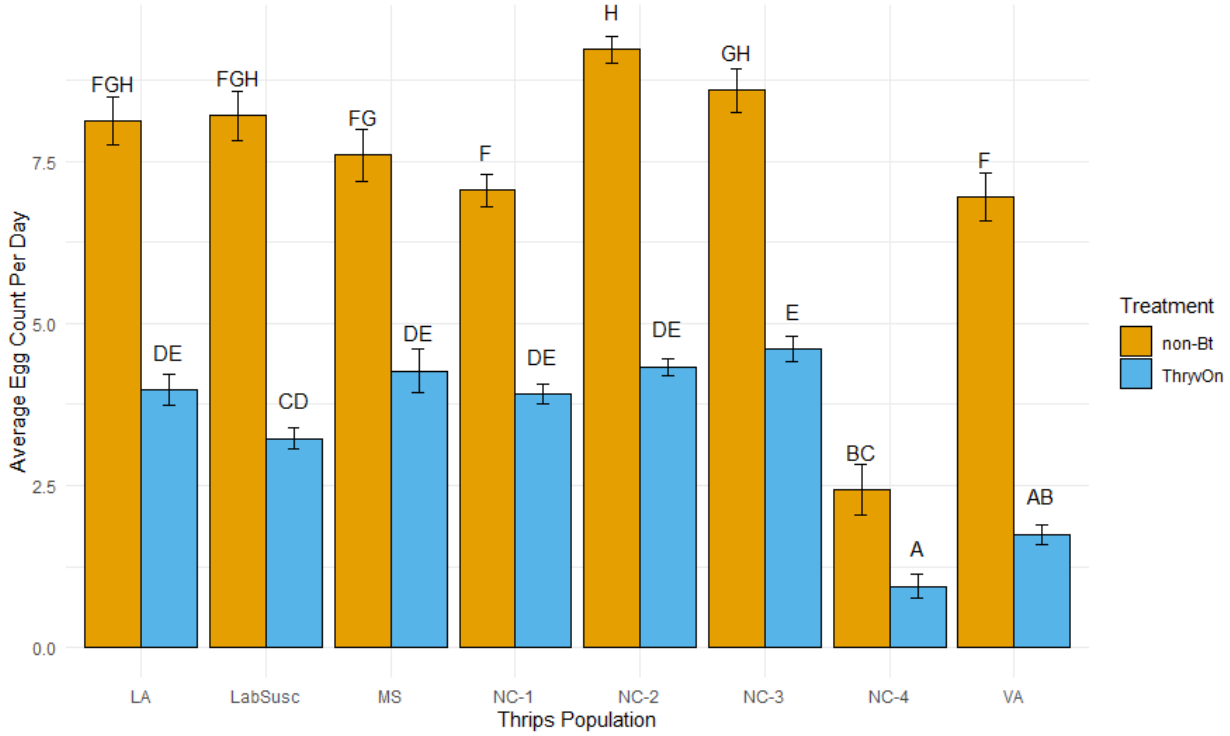
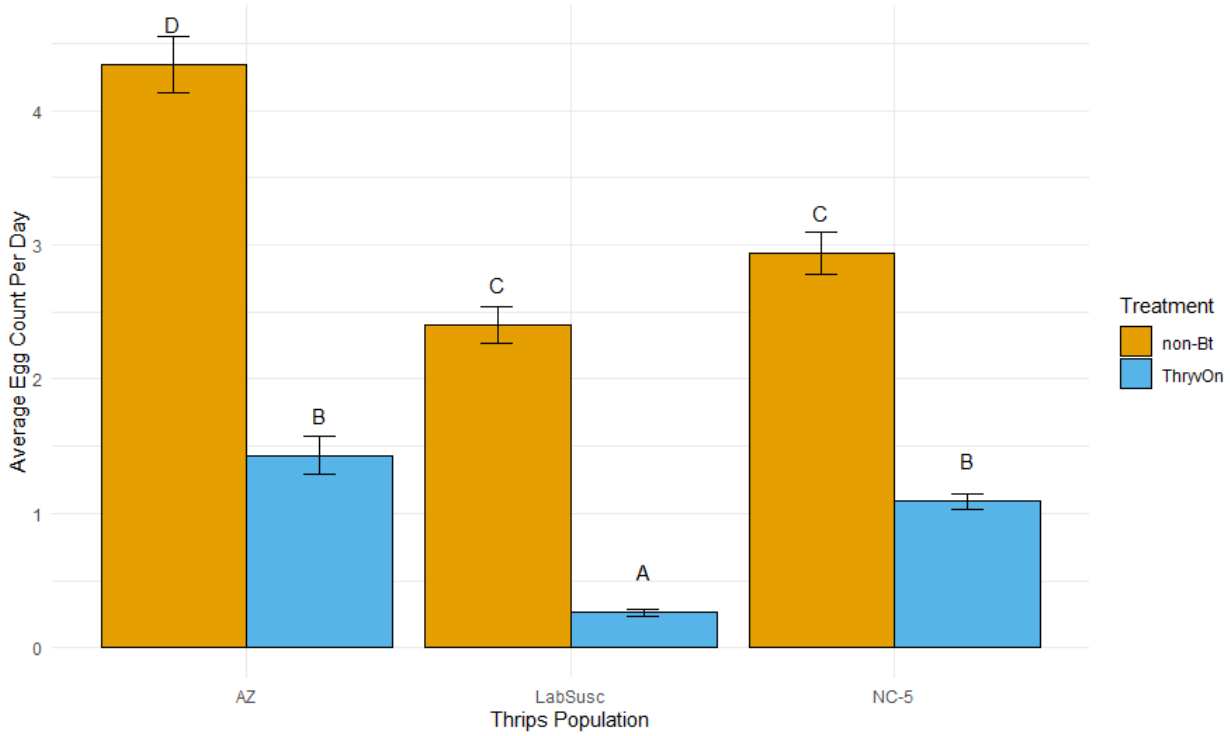


Figure 2.4: Average number of eggs per *F. occidentalis* female per day separated by location and treatment, with standard error. A Tukey's HSD on the estimated marginal means of the interaction between treatment and location denotes significance by letters.



Chapter 3:

Impacts of Foliar Insecticides on Natural Enemy Communities in Cotton

Abstract: Cotton hosts a variety of agricultural pests, many of which require pesticide interventions to limit yield loss. However, insecticides may undermine biological control services provided by the natural enemy communities also found in cotton. In this multiyear field study, we measured the effects of common lepidopteran and hemipteran insecticides on natural enemy communities in cotton. Across multiple states, we used standardized protocols to apply insecticides during the first week of bloom in each year. We then sampled natural enemy communities using sweep nets to track changes in community composition over four weeks. In addition to analyzing overall change, we focused a second analysis on two important generalist predators: *Geocoris* spp. and *Orius insidiosus* (Say). These species were selected because of their role in controlling pests and the potential for added sensitivity to insecticides targeting a taxonomically similar pest, *Lygus lineolaris* (Palisot de Beauvois). Across seven locations, natural enemy communities exposed to methoxyfenozide, chlorantraniliprole, sufloxaflor, novaluron, spinetoram, and bifenthrin were not significantly different from the control plots. However, the broad-spectrum insecticide acephate was associated with a decrease in natural enemy abundance. This result aligns with prior work documenting the negative effects of broad-spectrum organophosphate insecticides on natural enemies. Remarkably, the combination of acephate plus novaluron had greater overall natural enemy abundance when compared to the acephate only in all our studies. Overall, the study showed natural enemy communities were resilient to multiple insecticides but confirms that reduced risk insecticides may have fewer impacts to biological control services when compared to broad-spectrum materials.

Keywords: *Orius insidious*, natural enemies, *Gossypium hirsutum,* non-target impacts

Introduction

Upland cotton (*Gossypium hirsutum* L.) is infested by many different arthropod pests that feed on vegetative and reproductive tissues. As a result, farmers depend on insect protection from synthetic insecticides and genetically engineered varieties that express *Bacillus thuringiensis* (*Bt*) toxins. Despite improvements in control technologies, arthropod pests caused an estimated 4.2% yield reduction in the United States in 2020 (Cook and Threet, 2021). At a global scale, roughly 18% of the world's insecticidal use is used to produce cotton (Deguine *et al.,* 2008) and, because of widespread use, cotton insecticides may have considerable unanticipated effects on farm workers, rural communities, and the environment. Integrated pest management (IPM) is a framework designed to alleviate reliance on pesticide inputs using a combination of biological, cultural, and chemical control strategies to suppress insect pest populations (Kogan, 1998). Preserving natural enemies is an important component of the IPM framework but can be overlooked when balancing immediate needs for pest control (Ruberson *et al.,* 1998; Sansone and Smith, 2001; Berry & Mansfield *et al.,* 2006).

Surveys of cotton arthropod communities have reported 300 to 600 different species, of which only a dozen are significant pests (Deguine *et al.,* 2008). In the United States, the most common pests of cotton not currently controlled by *Bt* varieties are bollworm (*Helicoverpa zea* Boddie), plant bugs (*Lygus lineolaris* Palisot de Beauvois, *Lygus hesperus* Knight), and thrips (*Frankliniella fusca* Hinds, *Frankliniella occidentalis* Pergrande) (Cook and Threet, 2020). Of these, predation of lepidopteran eggs and larvae has been widely studied, with hemipteran and coleopteran predators providing the majority of biological control services (Nuessly and Sterling, 1994; Sanson and Smith; 2001; Pfannenstiel and Yeargan, 2002; Head *et al.,* 2005). Key predators of lepidopteran eggs and larvae include minute pirate bug (*Orius* spp.), big eyed bugs

(*Geocoris* spp.), and ladybird beetles (Coccinellidae) (Nuessly and Sterling, 1994; Sansone and Smith, 2001; Torres and Ruberson, 2006; Peterson *et al.,* 2018). The efficacy of generalist predators as biological control agents has been debated because they are prone to intraguild predation, however, many researchers believe these predators can effectively suppress pests of cotton (Symondson *et al.,* 2002). For example, a previous study of biological control in cotton documented that 74-81% of *H. zea* mortality could be attributed to *Orius* spp. (Sansone and Smith, 2001). Given that 11.7 million acres of cotton are planted in the United States, preservation of arthropod communities that provide biological control services is clearly part of the solution to lessen insecticide use at large scales to develop a more sustainable and resilient production system (USDA NASS, 2020).

From the pest management perspective, insecticide resistance is one major challenge that can drive periodic insecticide use increases in response to problematic pest populations. As a result, resistance presents both challenges and opportunities for sustainable biological control in cotton (Ehler, 1998). Because natural enemies are one form of non-chemical control, biological control services may represent an important, but complex role in Insecticide Resistance Management (IRM) (Onstad *et al.,* 2014). For example, biological control may either accelerate or decelerate resistance depending on site-specific ecological interactions between predator and prey (Gould *et al.,* 1991; Liu *et al.,* 2014); however, the consensus is that preservation of natural enemy communities provides benefits to agriculture (Onstad *et al.,* 2014). To sustain biological control services within agricultural fields, deliberate selection of insecticides based on non-target toxicity is one important step to limit negative impacts to beneficial insect communities.

In the past, the use of broad-spectrum insecticides (e.g., organophosphate, carbamate) had well documented negative impacts on natural enemies and, in turn, biological control services

limiting pest population development (Theiling and Croft, 1988). Recent advancements in pesticide specificity have reduced acute toxicity to natural enemies, a process that has enabled a more effective integration of biological and chemical control strategies in many crops (Gentz *et al.,* 2010). However, indirect effects on natural enemies are not well understood and may have unanticipated impacts on the longevity, fecundity or fertility, mobility, emergence rates, development time, or behavior of natural enemies (Cloyd, 2012). Moreover, insecticides can have subtle effects, causing a combination of direct and indirect disruptions across agricultural crop food webs (Elzen, 2001; Tooker and Pearsons, 2021). For example, omnivorous natural enemies can be directly exposed to insecticides during facultative herbivory stages of their life cycle (Albajes and Alomar, 1999). Indirectly, toxicants can be accumulated from prey to predator resulting in cascading impacts on the arthropod food web that are not obvious immediately following insecticidal exposure (Ruberson *et al.,* 1998).

Currently there are insecticides with differing modes of action registered for use in cotton (Alabama Extension, 2021; NC State Extension, 2022; SC Extension, 2022; UGA Extension, 2022; Virginia Extension, 2022). In other cropping systems, researchers have documented varied effects of common cotton insecticides on the composition and function of natural enemy communities (Douglas *et al*., 2015; Fernandes *et al.,* 2016; Hill *et al.,* 2017; Regan *et al.,* 2017; Siviter and Muth, 2020; Granados-Echegoyen *et al.,* 2021; Lytle *et al.,* 2021). Because generalist predators can be abundant in *Bt* cotton fields, an assessment of the non-target effects for these insecticides will be useful information to help growers balance efficacy on secondary pests with toxicity to natural enemies (Naranjo 2005; Cattaneo *et al.,* 2006; Romeis *et al.,* 2006; Ali *et al.,* 2016). Furthermore, the taxonomic similarities between hemipteran predators and *Lygus lineolaris* increases concern that insect growth regulator insecticides (i.e., novaluron; Diamond®,

Makhteshim Agan of North America – ADAMA, Raleigh, NC) may have unanticipated effects on specific taxa within the natural enemy populations. To address this knowledge gap, we used large-scale field trials across multiple North Carolina and Virginia locations to assess the non-target impacts of multiple insecticidal treatments with different modes of action on beneficial insect communities found in cotton. To determine the acute and/or chronic effects of acephate, we conducted a complementary regional field trial in 2021 to gather more information across a greater geographical scale. We hypothesized that broad spectrum organophosphate insecticides (i.e., acephate) would significantly reduce the total abundance of natural enemies when compared to reduced-risk organophosphate-alternative insecticides. We also hypothesized that the insect growth regulator, novaluron, would significantly reduce the number of omnivorous hemipteran predators due to known activity against a similar pest species, *Lygus lineolaris.* Together, results of this study help to bridge the knowledge gap of how reduced-risk insecticides may negatively impact biological control services in the contemporary cotton production system.

Materials and Methods

Reduced-Risk Insecticide Screening Study

To evaluate the non-target impacts of common active ingredient use in cotton, field experiments were conducted in North Carolina and Virginia during the 2020 and 2021 growing seasons (N=4 locations in each year) (Table 3.1). In 2020, plots were four row wide by 15.2 m long with seven treatments and an untreated control arranged in a randomized complete block design with four replications (Table 3.1). In 2021, we increased plot size to eight rows wide by 22.9–30.5 m limit inter-plot natural enemy dispersal. We also reduced the 2021 study to four treatments and an untreated control to focus our question on specific modes of action (Table 3.1). All study locations were planted between May and early June depending on weather conditions.

All insecticides included were representative of common active ingredients and formulations used in southeastern U.S. cotton production (Alabama Extension, 2021; NC State Extension, 2022; SC Extension, 2022; UGA Extension, 2022; Virginia Extension, 2022).

Insecticides were applied during the second week of bloom in late-July or early-August. The method of application was different among field trials based on the equipment available at each research station; however, the rate of formulated product was equivalent across study locations (Table 3.1, S3.1). To assess the composition of natural enemy communities among locations, pre-spray samples were taken to determine the baseline abundance and composition. Following spray application, we sampled each study location at five time points (3, 7, 14, 21, 28 days after treatment). In 2021, the 28-day sample was eliminated to enable an expansion in study locations. Additionally, the preliminary analysis of 2020 data indicated that the 28-day time point had no treatment separation due to natural enemy recolonization and population resurgence. Samples were taken using a standard sweep net (38 cm diameter) in sweeps across one row sweeping pattern across the top of the canopy totaling 25 sweeps from the center of each plot to avoid edge effects (Kogan & Pitre, 1980). Plot samples placed in 3.78 L Ziploc® bags (S.C. Johnson & Son, Racine, WI) and stored in a -20°C freezer. In the laboratory, all arthropod predators and spiders were separated from frozen plant foliage and identified to genus or species for analysis.

Acephate Toxicity Study

To determine the acute and chronic effects of acephate, we conducted a regional field trial in 2021 to gather more information across a greater geographical scale to better understand the severity of acephate use on natural enemy communities. The experimental design was identical to the larger field trial but only included two insecticide treatments and a control (Table 3.1). The

same study was replicated in Prattville, Alabama; Jackson Springs, North Carolina; and Blackville, South Carolina (S3.1). Samples were collected from pre-spray, 3, 7, 14, and 21 days after treatment. Samples were frozen and shipped overnight to North Carolina State University for identification and analysis.

Statistical Analysis

To determine differences in natural enemy communities among locations, we tested the pre-spray data for site-wise differences in total natural enemy abundance using a one-way ANOVA in R (Version 1.3.1093, R Core Team, 2020). The univariate ANOVA location analyses detected significant differences in total natural enemy abundance among locations prior to insecticide treatment ($P < 0.05$, Tables 3.4). As a result, location was included as a fixed effect in each analysis to account for study site variation. The Reduced-Risk Insecticide Screening Study and Acephate Toxicity Study were analyzed with repeated measures linear mixed models (PROC GLIMMIX, SAS Institute Inc. 2016) in SAS (Version 3.8 Enterprise Edition, SAS Institute, Cary, NC, USA). Given the experimental design differences among studies, the collected data were divided into three separate analyses, the Reduced-Risk Insecticide Screening Study for years 2020, and 2021, and the Acephate Toxicity Study for 2021 (Table 3.1). Two response variables were analyzed for each study: 1) all beneficial insects and spiders (Order: Araneae); 2) a subset of the dataset only including omnivorous generalist predators (Families: Geocoridae and Anthocoridae). Main effects for location, treatment, days after treatment (DAT), and an interaction of treatment by DAT were included as fixed effects in the model. Random effects of block were added to all models. The models were fitted to a Poisson distribution, and compound symmetry covariance structures were used to model the effects of the repeated measures. If a significant fixed effect was detected ($P < 0.05$), a Tukey's test was used for means separation. In

the event of a significant interaction ($P < 0.05$), the SLICE, SLICEDIFF, and SLICEBYTYPE functions (PROC GLIMMIX, SAS Institute Inc. 2016), were used to compare the treatments to the untreated controls on individual sampling dates (DAT).

Results

Reduced-Risk Insecticide Screening Study

In 2020, we sampled a total of 4724 individual natural enemy arthropods from 52 families across locations. Omnivorous hemipterans were the dominant group in 2020, comprising 56% of the counted total beneficial species, including spiders. The second largest group was Coccinellidae representing 24% of sampled arthropods. In the 2020 results of all beneficials and the hemipterans only subset, the interaction of treatment and days after treatment (DAT) was not significant (Table 3.2). In both analyses, the main effects for location and DAT were significant, and the main effects of treatment were not significant (Table 3.2). No significant differences were observed between the number of natural enemies in the insecticide treatment plots and the control plots.

In 2021, we sampled a total of 1,929 individual natural enemies in 24 families across locations. Omnivorous hemipterans were the dominant taxa in the second year, comprising 62% of the sampled beneficial insects. The second largest group was coccinellid beetles, at 21%. In the analysis of all beneficial insects and spiders for 2021, we detected a significant interaction between treatment and DAT (Table 3.3). When the interaction was sliced by DAT with multiple comparisons, acephate significantly reduced arthropod numbers when compared to the untreated control on days three ($t = -3.77$; $df = 45$; $P < 0.001$) and seven ($t = -3.37$; $df = 45$; $P = 0.002$). Interestingly, the treatment pairing of acephate and novaluron was not significantly different compared to the untreated control on any DAT. The interaction of DAT and treatment was not

significant for omnivorous hemipteran beneficial subset in 2021, however, the fixed effect of treatment was significant (Table 3.3). Overall, acephate had a significantly lower number of beneficial insects compared to the control (Figure 3.1). The results from the main effect of treatment in the omnivorous hemipterans showed that the mix of acephate and novaluron did not significantly differ from the untreated control (Figure 3.1).

Acephate Toxicity Study

The most prominent beneficial group in the regional trial was the hemipteran omnivores at 62% of the total natural enemy collection. Coccinellidae beetles and ant-like flower beetles (Family: Anthicidae) comprised the successive groups equally at approximately 13% of total abundance. Significant differences in the total natural enemies were observed among insecticides treatments (Table 3.4). Acephate significantly reduced the number of natural enemies in the regional study (Figure 3.2). However, the combination of acephate and novaluron had no significant effect on total natural enemy abundance relative to the control. There was not a significant treatment effect on the abundance of hemipteran omnivores, suggesting that activity of acephate may have a larger effect on minor groups (Table 3.4). DAT were significant in both datasets, but the interaction between treatment and DAT were not significant in either.

Discussion

In this multi-year study, we evaluated common cotton insecticides for non-target impacts on natural enemy populations under realistic conditions. The 2020 Reduced-Risk Insecticide Screening Study suggests that the foliar insecticides did not have a strong negative impact on natural enemy populations after spraying; despite the average number of beneficial insects per sample being slightly lower in the treated plots at 3 DAT. We did detect marginally more beneficial arthropods in the standalone novaluron insecticide treatment (Figure 3.3). Novaluron

(Diamond®) is an insect growth regulator that targets *Lygus* spp. and Lepidoptera and previous research found minimal detrimental effects to the common natural enemies found in our study, like spiders or lady beetles (Maxwell and Fadamiro, 2006). In our study, the selected insecticides did not negatively impact the beneficial insect populations in 2020, nor did they impact omnivorous hemipterans in a separate analysis. This result is important because several of these insecticides are critical components for cotton IPM programs. However, previous literature does report that broad-spectrum insecticides, such as bifenthrin, have well documented negative effects on beneficial communities suggesting that this insecticide may not be well-suited for some integrated pest management programs (Boyd and Boethel, 1998; Elzen *et al.,* 1998; Balakrishnan *et al.,* 2009).

In 2021, acephate caused significant declines on total natural enemy populations in the Reduced-Risk Insecticide Screening Study and the Acephate Toxicity Study. This reaffirms previous literature that broad-spectrum organophosphates can have negative effects on natural enemy communities (Abdelrahman & Munir, 1989; Naranjo, 2001). Despite causing numerical declines, the tank mix of acephate and novaluron was not significantly different from the control plots, and generally had higher natural enemies than the acephate only plot. Moreover, days after treatment (DAT) was significant in all analyses except for the beneficial insects and spiders in the 2021 Reduced-Risk Insecticide Screening Study ($P = 0.0504$). The short persistence of the selected insecticides in concert with dispersal of beneficial arthropods into plots shortly after application may explain the significant DAT effect. This trend was observed in all treatments including the untreated control. This result suggests that the period of natural enemy disruption with these insecticides was relatively short in our studies. It is important to note that our plot sizes were small when compared to the size of commercial cotton fields in our region. In large

commercial fields disturbance with broad-spectrum insecticides (i.e. like spraying acephate), may have lasting impacts on natural enemy communities. Although many factors influence recolonization, the severity of field-scale disturbance will depend on dispersal capability of the natural enemy taxa and the size of populations residing in non-crop areas that serve as a reservoir. An alternative explanation for the population fluctuations growth observed in all treatments, including the untreated controls, may be attributed to increasing prey availability through the cotton reproductive cycle. As the number of cotton blooms increase, the attractiveness of the crop has positive effects on the prey population and, in turn, the development of natural enemy populations (Figures 3.3-3.6)(Eklassabany *et al.,* 1995; Symondson *et al.,* 2002). Moreover, predator and parasitoid populations may increase due to availability of extrafloral nectaries in cotton (Wäckers *et al.,* 2001; Wäckers & Bonifay, 2004; Hagenbucher *et al.,* 2013; Llandres *et al.,* 2019).

Furthermore, location was significant in all studies except for 2021 hemipterans-only (P=0.079). It is important to note that, our methodology was the standardized across all locations, however, there may have been differences in sampling efforts across collaborators. While sampling bias may have accounted some of the variability across locations, each location had the same researcher sample for the entirety of the study. Alternatively, differences in natural enemy abundance among locations could be driven by surrounding landscape composition which have been connected to biological control services in other studies (Bianchi *et al.,* 2006; Chaplin-Kramer *et al.,* 2011).

The emergence of *Bt* resistant *Helicoverpa zea* populations are a new concern for a large portion of the U.S. Cotton Belt (Reisig *et al.,* 2018; Kerns *et al.,* 2022). As the frequency of *Bt* resistant *Helicoverpa zea* populations increase (Niu *et al.,* 2021; Yang *et al.,* 2021; Yu *et al.,*

2021), preservation of natural enemies will become an increasingly important component of the system because biological control services can help suppress problematic populations (Machado *et al.,* 2019; Bordini *et al.,* 2021). Here, we monitored direct impacts on beneficial communities following applications of insecticides in an effort to document which active ingredients best complement biological control services with minimal non-target impacts. One major limitation of our experimental design is the absence of pest predation data following sprays. Linking changing species abundance with actual impacts on pest predation is an important to verify how different spray decisions affect biological control services. Furthermore, estimating the efficacy of insecticides on target pests in tandem with biological control is anotehr critical piece of information to help farmers make balanced decisions. Despite this limitation, our study is meaningful because we observed minimal negative effects for most insecticides across different locations and insecticide application equipment. The generality of this non-effect was striking, with the acute reductions occurring only in acephate treatments. Interestingly, treatments combining novaluron and acephate did not separate from the untreated control plots in our study. It is unclear why tank mixes that included acephate did not have the same or greater suppressive effects when compared to acephate alone. We also observed limited negative effects of early to mid-season insecticides (i.e., sulfoxaflor and chlorantraniliprole) that are commonly used in cotton during the bloom stage when natural enemy communities begin increasing.

In summary, this study collected field-relevant information about how several common insecticides interact with natural enemy communities in the context of a contemporary *Bt* cotton production system. Our results showed that beneficial insect communities are more resilient than we expected, with few significant impacts, implying that reduced-risk insecticides could be more compatible with biological control services than previously thought. Future studies should

explore the effect of selected insecticides (particularly acephate) at the scale of commercial cotton fields where insecticides may more effectively deplete natural enemy communities. Moreover, targeted examination of changing species diversity in response to insecticides at the local (alpha diversity) and regional scale (beta diversity) may reveal additional insight into the sensitivity of specific species within the natural enemy community. Additionally, analysis of species composition in untreated plots throughout the season will also provide meaningful baseline information about natural enemy community variation found in cotton across the southeast region. This information will be important as the production practices continue to change, especially with the release of the first *Bt* trait targeting hemipteran and thysanopteran pests (i.e., ThryvOn, expressing Cry51Aa2.834_16, Bayer Crop Science) (Bachman *et al*., 2017; Akbar *et al*., 2019). Together these baseline assessments can be leveraged to make better recommendations to growers that balance efficacy against target pests with biological control services in cotton.

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References

Abdelrahman, A.A., Munir, B., (1989). Sudanese experience in integrated pest management of cotton. *International Journal of Tropical Insect Science*. **10**(6), 787–794.

Alabama Extension (2021). Cotton. Insect, Disease, Nematode and Weed Recommendations for 2021. Alabama A&M and Auburn Universities Cooperative Extension Service [Online]. https://www.aces.edu/wp-content/uploads/2019/10/IPM-0415_Cotton_2021_121120L-G.pdf [Accessed April 21st, 2022].

Albajes, R., & Alomar, Ò. (1999). Current and potential use of polyphagous predators. In Albajes, R., Lodovica Gullino, M., van Lenteren, J.C., Elad, Y. (eds) *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 265-275). Springer, Dordrecht.

Ali, A., Desneux, N., Lu, Y., Liu, B., & Wu, K. (2016). Characterization of the natural enemy community attacking cotton aphid in the *Bt* cotton ecosystem in Northern China. *Scientific Reports*, **6**(1), 1-9.

Akbar, W., Gowda, A., Ahrens, J.E., Stelzer, J.W., Brown, R.S., Bollman, S.L., Greenplate, J.T., Gore, J., Catchot, A.L., Lorenz, G., Stewart, S.D., Kerns, D.L., Greene, J.K., Toews, M.D., Herbert, D.A., Reisig, D.D., Sword, G.A., Ellsworth, P.C., Godfrey, L.D. and Clark, T.L. (2019). First transgenic trait for control of plant bugs and thrips in cotton. *Pest. Management Science*, **75,** 867-877.

Bachman, P.M., Ahmad, A., Ahrens, J.E., Akbar, W., Baum, J.A., Brown, S., Clark, T.L., Fridley, J.M., Gowda, A., Greenplate, J.T., Jensen, P.D., Mueller, G.M., Odegaard, M.L., Tan, J., Uffman, J.P., & Levine, S. L. (2017). Characterization of the activity spectrum of MON 88702 and the plant-incorporated protectant Cry51Aa2. 834_16. *PLoS One*, **12**(1), e0169409.

Balakrishnan, N., Kumar, B.V., & Sivasubramanian, P. (2009). Bioefficacy of bifenthrin 10 EC against sucking insects, bollworms and natural enemies in cotton. *Madras Agriculture Journal*, **96**(1-6), 225-229.

Berry, J.A., & Mansfield, S. (2006). Hyperparasitoids of the gum leaf skeletoniser, *Uraba lugens* Walker (Lepidoptera: Nolidae), with implications for the selection of a biological control agent for *Uraba lugens* in New Zealand. *Australian Journal of Entomology*, **45**(3), 215-218.

Bianchi, F. J., Booij, C. J. H., & Tscharntke, T. (2006). Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B: Biological Sciences*, **273**(1595), 1715-1727.

Boyd, M.L., & Boethel, D.J. (1998). Susceptibility of predaceous hemipteran species to selected insecticides on soybean in Louisiana. *Journal of Economic Entomology*, **91**(2), 401-409.

Bordini, I., Ellsworth, P.C., Naranjo, S.E., & Fournier, A. (2021). Novel insecticides and generalist predators support conservation biological control in cotton. *Biological Control*, **154**, 104502.

Cattaneo, M. G., Yafuso, C., Schmidt, C., Huang, C. Y., Rahman, M., Olson, C., Ellers-Kirk, C., Orr, B., Marsh, S., Antilla, L., Dutilleul, P., & Carrière, Y. (2006). Farm-scale evaluation of the impacts of transgenic cotton on biodiversity, pesticide use, and yield. *Proceedings of the National Academy of Sciences*, **103**(20), 7571-7576.

Chaplin-Kramer, R., O'Rourke, M. E., Blitzer, E. J., & Kremen, C. (2011). A meta-analysis of crop pest and natural enemy response to landscape complexity. *Ecology letters*, **14**(9), 922-932.

Cloyd, R. A. (2012). Indirect effects of pesticides on natural enemies. In: Soundararajan, R.P. (ed) *Pesticides—advances in chemical and botanical pesticides*, (pp. 126-130). Rijeka, Croatia: Intech.

Cook, W., and Threet, M. (2020). Cotton insect losses [Online]. Mississippi State University. https://www.biochemistry.msstate.edu/resources/pdf/2020/table28.pdf [Accessed: April 15th 2022].

Deguine, J. P., Ferron, P., & Russell, D. (2008). Sustainable pest management for cotton production. A review. *Agronomy for Sustainable Development*, **28**(1), 113-137.

Dorman, S. J., Gross, A. D., Musser, F. R., Catchot, B. D., Smith, R. H., Reisig, D.D., Reay-Jones, F., Greene, J., Roberts, P., & Taylor, S. V. (2020). Resistance monitoring to four insecticides and mechanisms of resistance in *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae) populations of southeastern USA cotton. *Pest Management Science*, **76**(12), 3935-3944.

Douglas, M. R., Rohr, J. R., & Tooker, J. F. (2015). Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield. *Journal of applied ecology*, **52**(1), 250-260.

Ehler, L. (1998). Conservation biological control: past, present, and future. In: Barbosa, P. (ed) *Conservation Biological Control* (pp. 1-8). Academic Press. San Diego.

Elkassabany, N., Ruberson, J. R., & Kring, T. J. (1996). Seasonal distribution and overwintering of Orius insidiosus (Say) in Arkansas. *Journal of Entomological Science*, **31**(1), 76-88.

Elzen, G.W., Elzen, P.J., King, E.G., (1998). Laboratory toxicity of insecticide residues to *Orius insidiosus*, *Geocoris punctipes*, *Hippodamia converge*ns, and *Chrysoperla carnea*. S*outhwestern Entomologist*, **23**, 335–342.

Elzen, G. W. (2001). Lethal and sublethal effects of insecticide residues on *Orius insidiosus* (Hemiptera: Anthocoridae) and *Geocoris punctipes* (Hemiptera: Lygaeidae). *Journal of Economic Entomology*, **94**(1), 55-59.

Fernandes, M. E., Alves, F. M., Pereira, R. C., Aquino, L. A., Fernandes, F. L., & Zanuncio, J. C. (2016). Lethal and sublethal effects of seven insecticides on three beneficial insects in laboratory assays and field trials. *Chemosphere*, **156**, 45-55.

Gentz, M. C., Murdoch, G., & King, G. F. (2010). Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. *Biological Control*, **52**(3), 208-215.

Gould, F., Kennedy, G., Johnson, M., (1991). Effects of natural enemies on the rate of herbivore adaptation to resistant host plants. *Entomologia experimentalis et Applicata*, **58**(1), 1-14.

Granados-Echegoyen, C., Loera-Alvarado, G., Miranda-Salcedo, M. A., Hernández-Cruz, J., Luna-Cruz, A., & Loera-Alvarado, E. (2021). Field efficacy of synthetic and botanical-derived insecticides against *Melanaphis sacchari*, and non-target and beneficial species associated with cultivated sorghum. *Southwestern Entomologist*, **46**(1), 33-46.

Hagen, K. S., & van den Bosch, R. (1968). Impact of pathogens, parasites, and predators on aphids. *Annual Review of Entomology*, **13**(1), 325-384.

Hagenbucher, S., Olson, D. M., Ruberson, J. R., Wäckers, F. L., & Romeis, J. (2013). Resistance mechanisms against arthropod herbivores in cotton and their interactions with natural enemies. *Critical Reviews in Plant Sciences*, **32**(6), 458-482.

Head, G., Moar, W., Eubanks, M., Freeman, B., Ruberson, J., Hagerty, A., & Turnipseed, S. (2005). A multiyear, large-scale comparison of arthropod populations on commercially managed *Bt* and non-*Bt* cotton fields. *Environmental Entomology*, **34**(5), 1257-1266.

Hill, M. P., Macfadyen, S., & Nash, M. A. (2017). Broad spectrum pesticide application alters natural enemy communities and may facilitate secondary pest outbreaks. *PeerJ*, **5**, e4179.

Kerns, Dawson D., Fei Yang, David L. Kerns, and Scott D. Stewart. 2022. Evaluation of *Bt* resistance in *Helicoverpa zea* (Lepidoptera: Noctuidae) strains using various *Bt* cotton plant tissues. *Pest Management Science* ,**78**(1): 95-103.

Kogan, M., & Herzog, D. C. (1980). *Sampling Methods in Soybean Entomology*. Springer-Verlag New York.

Kogan, M. (1998). Integrated pest management: historical perspectives and contemporary developments. *Annual Review of Entomology*, **43**(1), 243-270

Liu, X., Chen, M., Collins, H. L., Onstad, D. W., Roush, R. T., Zhang, Q., Earle, E., & Shelton, A. M. (2014). Natural enemies delay insect resistance to Bt crops. *PloS One*, **9**(3), e90366.

Llandres, A.L., Verdeny-Vilalta, O., Jean, J., Goebel, F.R., Seydi, O., & Brévault, T. (2019). Cotton extrafloral nectaries as indirect defense against insect pests. *Basic and Applied Ecology*, **37**, 24-34.

Lytle, A.J., & Huseth, A.S. (2021). Impact of foliar insecticide sprays on Melanaphis sacchari (Hemiptera: Aphididae) and natural enemy populations in grain sorghum. *Crop Protection*, **149**, 105764.

Machado, A.V., Potin, D.M., Torres, J.B., & Torres, C.S.S. (2019). Selective insecticides secure natural enemies action in cotton pest management. *Ecotoxicology and Environmental Safety*, **184**, 109669.

Maxwell, E.M., & Fadamiro, H.Y. (2006). Evaluation of several reduced-risk insecticides in combination with an action threshold for managing lepidopteran pests of cole crops in Alabama. *Florida Entomologist*, **89**(2), 117-126.

Naranjo, S.E. (2001). Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. *Crop Protection*, **20**(9), 835-852.

Naranjo, S.E. (2005). Long-term assessment of the effects of transgenic *Bt* cotton on the function of the natural enemy community. *Environmental Entomology*, **34**(5), 1211-1223.

NC State Extension (2022). North Carolina Agricultural Chemicals Manual. North Carolina Cooperative Extension Service. [Online] https://content.ces.ncsu.edu/north-carolina-agricultural-chemicals-manual [Accessed April 17th, 2022].

Niu, Y., Oyediran, I., Yu, W., Lin, S., Dimase, M., Brown, S., ... & Huang, F. (2021). Populations of *Helicoverpa zea* (Boddie) in the southeastern United States are commonly resistant to Cry1Ab, but still susceptible to Vip3Aa20 expressed in MIR 162 corn. *Toxins*, 13(1), 63.

Nuessly, G.S., & Sterling, W.L. (1994). Mortality of *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs in cotton as a function of oviposition sites, predator species, and desiccation. *Environmental Entomology*, **23**(5), 1189-1202.

Onstad, D.W., Shelton, A.M., & Flexner, J.L. (2014). Insecticide resistance, natural enemies, and density-dependent processes. *In* D.W. Onstad *Insect Resistance Management: Biology, Economics, And Prediction***.** Elsevier, Boston, USA. pp. 403-419.

Peterson, J.A., Burkness, E.C., Harwood, J.D., & Hutchison, W.D. (2018). Molecular gut-content analysis reveals high frequency of *Helicoverpa zea* (Lepidoptera: Noctuidae) consumption by *Orius insidiosus* (Hemiptera: Anthocoridae) in sweet corn. *Biological Control*, **121**, 1-7.

Pfannenstiel, R. S., & Yeargan, K. V. (2002). Identification and diel activity patterns of predators attacking *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs in soybean and sweet corn. *Environmental Entomology*, **31**(2), 232-241.

Reisig, D.D., Huseth, A.S., Bacheler, J.S., Aghaee, M.A., Braswell, L., Burrack, H.J., Flanders, K., Greene, J.K., Herbert, D.A., Jacobson, A., Paula-Moraes, S.V., Roberts, P., & Taylor, S.V. (2018). Long-term empirical and observational evidence of practical *Helicoverpa zea* resistance to cotton with pyramided *Bt* toxins. *Journal of Economic Entomology*, **111**(4), 1824-1833.

Regan, K., Ordosch, D., Glover, K. D., Tilmon, K. J., & Szczepaniec, A. (2017). Effects of a pyrethroid and two neonicotinoid insecticides on population dynamics of key pests of soybean and abundance of their natural enemies. *Crop Protection*, **98**, 24-32.

Romeis, J., Meissle, M., & Bigler, F. (2006). Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology*, **24**(1), 63-71.

Ruberson, J., Nemoto, H., & Hirose, Y. (1998). Pesticides and conservation of natural enemies in pest management. In: Barbosa, P. (ed) *Conservation biological control* (pp. 207-220). Academic Press. San Diego.

Sansone, C. G., & Smith, J. W. (2001). Natural mortality of *Helicoverpa zea* (Lepidoptera: Noctuidae) in short-season cotton. *Environmental Entomology*, **30**(1), 112-122.

SAS Institute Inc. 2010. SAS/STAT® 9.22 User's Guide (PROC GLIMMIX). Cary, NC. SAS Institute Inc.

SC Extension (2022). South Carolina Pest Management Handbook. South Carolina Cooperative Extension Service. [Online] https://www.clemson.edu/extension/agronomy/pestmanagment2022/2022pmhmaster.pdf [Accessed April 21st, 2022].

Siviter, H., & Muth, F. (2020). Do novel insecticides pose a threat to beneficial insects? *Proceedings of the Royal Society B*, **287**(1935), 20201265.

Symondson, W.O.C., Sunderland, K.D., & Greenstone, M.H. (2002). Can generalist predators be effective biocontrol agents? *Annual Review of Entomology*, **47**, 561**.**

Theiling, K. M., & Croft, B. A. (1988). Pesticide side-effects on arthropod natural enemies: a database summary. *Agriculture, Ecosystems & Environment*, **21**(3-4), 191-218.

Tooker, J.F., & Pearsons, K.A. (2021). Newer characters, same story: neonicotinoid insecticides disrupt food webs through direct and indirect effects. *Current Opinion in Insect Science*, **46**, 50-56.

Torres, J.B., & Ruberson, J.R. (2006). Interactions of *Bt*-cotton and the omnivorous big-eyed bug *Geocoris punctipes* (Say), a key predator in cotton fields. *Biological Control*, **39**(1), 47-57.

UGA Extension (2022). Cotton Insect Control. University of Georgia Extension Service [Online] https://extension.uga.edu/content/dam/extension/programs-and-services/integrated-pest-management/documents/handbooks/2022-comm-chapters/Cotton.pdf [Accessed April 21st, 2022].

USDA NASS (2020). United States Department of Agriculture National Agricultural Statistics Service. Acreage. [Online] https://www.nass.usda.gov/Publications/Todays_Reports/reports/acrg0621.pdf [Accessed 10 March, 2022].

Virginia Extension (2022). Insect Control in Field Crops. Virginia Cooperative Extension Service [Online]. https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/456/456-016/ENTO-461D.pdf [Accessed April 21st, 2022].

Wäckers, F. L., Zuber, D., Wunderlin, R., & Keller, F. (2001). The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor. *Annals of Botany*, **87**(3), 365-370.

Wäckers, F. L., & Bonifay, C. (2004). How to be sweet? Extrafloral nectar allocation by *Gossypium hirsutum* fits optimal defense theory predictions. *Ecology*, **85**(6), 1512-1518.

Whitcomb, W.H., & Bell, K.O. (1964). Predaceous insects, spiders, and mites of Arkansas cotton fields. *Arkansas Bulletin, Agricultural Experiment Station*. University of Arkansas, 690.

Yang, F., Kerns, D. L., Little, N. S., Santiago González, J. C., & Tabashnik, B. E. (2021). Early warning of resistance to *Bt* toxin Vip3Aa in *Helicoverpa zea*. *Toxins*, **13**(9), 618.

Yu, W., Lin, S., Dimase, M., Niu, Y., Brown, S., Head, G. P., ... & Huang, F. (2021). Extended investigation of field-evolved resistance of the corn earworm *Helicoverpa zea* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* Cry1A.105 and Cry2Ab2 proteins in the southeastern United States. *Journal of Invertebrate Pathology*, **183**, 107560.

Tables

Table 3.1 Insecticidal treatment list for the Reduced-Risk Insecticide Screening Study (RR) and Acephate Toxicity Study (AT).

Year	Study	Trade Name	Active Ingredient	Mode of Action Group (IRAC Group Number)	Rate formulated insecticide ha^{-1}	g (active ingredient $hectare^{-1}$
2020 2021	RR AT	Untreated control	-	-	-	-
2020	RR	Intrepid®	methoxyfenozide	Diacylhydrazines (18)	0.73 L	175.1
2020	RR	Radiant®	spinetoram	Spinosyns (5)	0.58 L	70.2
2020	RR	Intrepid Edge®	methoxyfenozide + spinetoram	Diacylhydrazines (18) + Spinosyns (5)	0.59 L methoxyfenozide + 0.59 L spinetoram	175.2 methoxyfenozide + 35.0 spinetoram
2020	RR	Diamond®	novaluron	Benzoylureas (15)	0.66 L	65.3
2020	RR	Brigade®	bifenthrin	Pyrethroids-Pyrethrins (3A)	0.47 L	112.1
2020 2021	RR	Prevathon®	chlorantraniliprole	Diamides (28)	1.023 L	52.9
2020 2021	RR	Transform®	sulfoxaflor	Sulfloximines (4C)	0.16 L	0.1
2021	RR AT	Orthene® 97	acephate	Organophosphates (1B)	560.4 g	545.8
2021	RR AT	Orthene® + Diamond®	acephate + novaluron	Organosphosphates (1B) + Benzoylureas (15)	560.4 g acephate + 0.66 L novaluron	545.8 acephate + 65.3 novaluron

Table 3.2 Repeated Measures Mixed Model with total number of natural enemies caught as the response for the Reduced-Risk Insecticide Screening Study in 2020. Hemipterans only is a subset of the full dataset that only includes *Orius insidiosus* and *Geocoris* spp. Asterisks represent statistical significance (*P < 0.05, **P < 0.01,***P < 0.001).

	Reduced-Risk Insecticide Screening Study 2020					
	Hemipterans Only			**All Beneficials and Spiders**		
	F	**df**	***P > F***	***F***	**df**	***P > F***
Location	47.10	3,109	0.000***	1.28	3,109	0.000***
DAT	31.98	4,128	0.000***	31.89	4,128	0.000***
Treatment	0.53	7,32	0.809	0.87	7,32	0.543
Treatment × DAT	1.27	28,128	0.187	31.25	28,128	0.177

Table 3.3 Repeated Measures Mixed Model with total number of natural enemies caught as the response for the Reduced-Risk Insecticide Screening Study in 2021. Hemipterans only is a subset of the full dataset comprised of *Orius insidiosus* and *Geocoris spp.*. Asterisks represent statistical significance (*P < 0.05, **P < 0.01,***P < 0.001).

	Reduced-Risk Insecticide Screening Study 2021					
	Hemipterans Only			**All Beneficials and Spiders**		
	F	**df**	***P>F***	***F***	**df**	***P>F***
Location	1.68	3,57	0.079	14.45	3,57	0.000***
DAT	2.95	3,45	0.042*	2.80	3,45	0.050
Treatment	4.82	4,15	0.011*	8.57	4,15	0.001***
Treatment × DAT	1.68	12,45	0.103	2.17	12,45	0.030*

Table 3.4 Repeated Measures Mixed Model with total number of beneficials caught as the response for the Acephate Toxicity Study. Hemipterans only is a subset of the full dataset comprised of *Orius insidiosus* and *Geocoris* spp. Asterisks represent statistical significance (*P < 0.05, **P < 0.01,***P < 0.001).

	Acephate Toxicity Study 2021					
	Hemipterans Only			**All Beneficials and Spiders**		
	F	**df**	***P > F***	***F***	**df**	***P > F***
Location	17.20	3,29	0.000***	25.18	3,29	0.000***
DAT	5.86	3,27	0.000***	8.32	3,27	0.000***
Treatment	4.82	2,9	0.149	6.60	2,9	0.017*
Treatment × DAT	1.33	6,27	0.414	1.88	6,27	0.120

Table 3.5 One-way ANOVA results of pre-spray natural enemies by location of the Reduced-Risk Insecticide Screening Study (RR) and Acephate Toxicity Study (AT) with pooled averages across sampled plots and Tukey's separation. Asterisks represent statistical significance (*P < 0.05, **P < 0.01,***P < 0.001). Locations marked with the same letter were not statistically different ($P < 0.05$).

Year	Study	*P>F*	Location	Mean ± Standard deviation
2020	RR	0.000***	Clayton	1.55 (0.23) b
			Goldsboro	1.50 (0.22) b
			Plymouth	3.16 (0.33) a
			Suffolk	2.90 (0.31) a
2021	RR	0.000***	Clayton	10.60 (0.94) a
			Goldsboro	8.20 (0.87) a
			Suffolk	4.50 (0.60) b
2021	AT	0.000***	Prattville	8.25 (5.09) b
			Blackville	5.08 (3.40) b
			Jackson Springs	24.00 (10.72) a

Figures

Figure 3.1 Omnivorous insect abundance from the Reduced-Risk Insecticide Screening Study across sampling locations for 2021. Treatments marked with the same letter were not statistically different ($P < 0.05$).

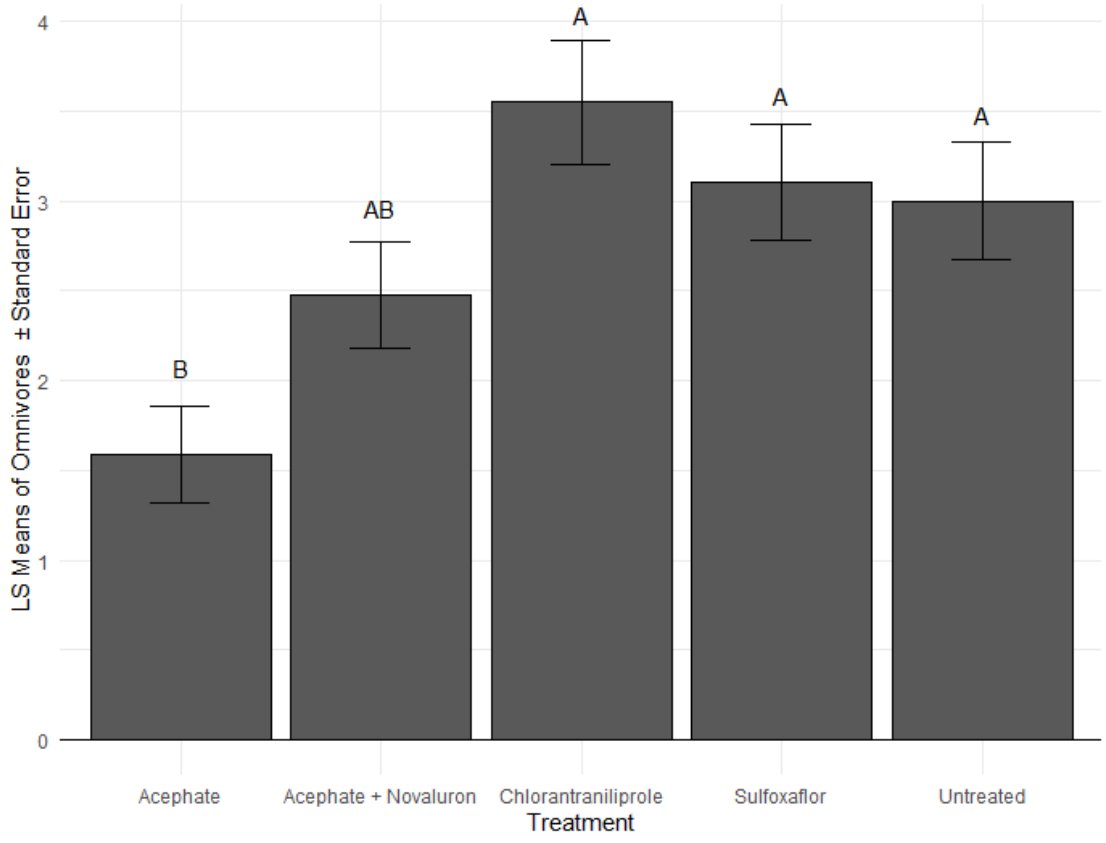


Figure 3.2 Natural enemy abundance displayed from the Acephate Toxicity Study in 2021 across sampling locations. Significance is denoted by varying letters with Tukey's adjustment. Treatments marked with the same letter were not statistically different ($P < 0.05$).

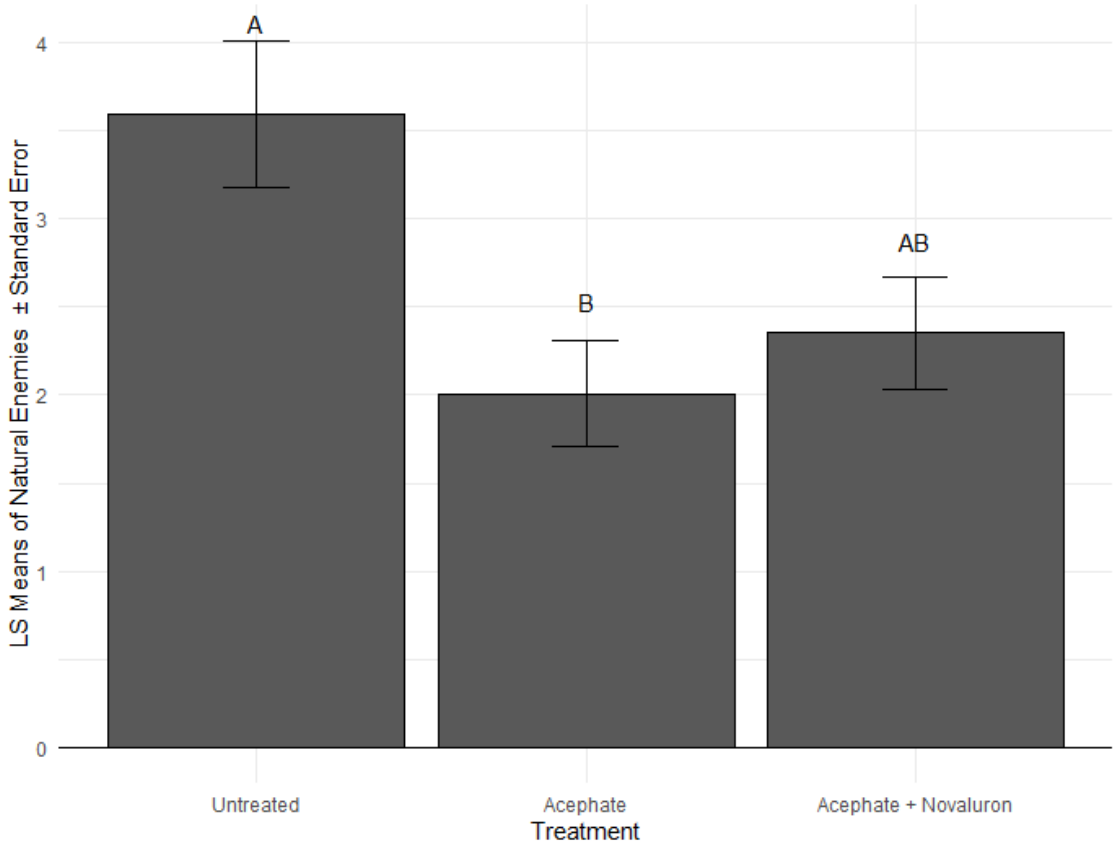


Figure 3.3 Average natural enemy count by treatment pooled across locations across four study locations in the 2020 Reduced-Risk Insecticide Screening Study.

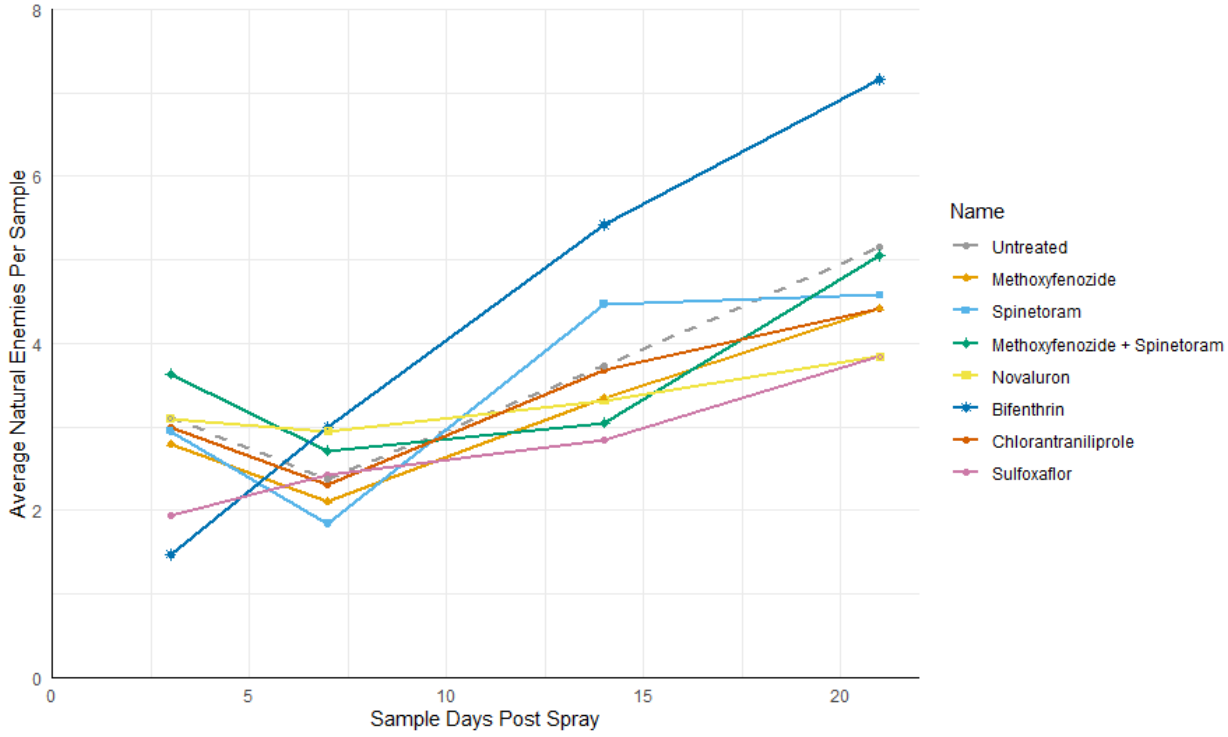


Figure 3.4 Average natural enemy caught by treatment over time pooled across four study locations in the 2021 Reduced-Risk Insecticide Screening Study.

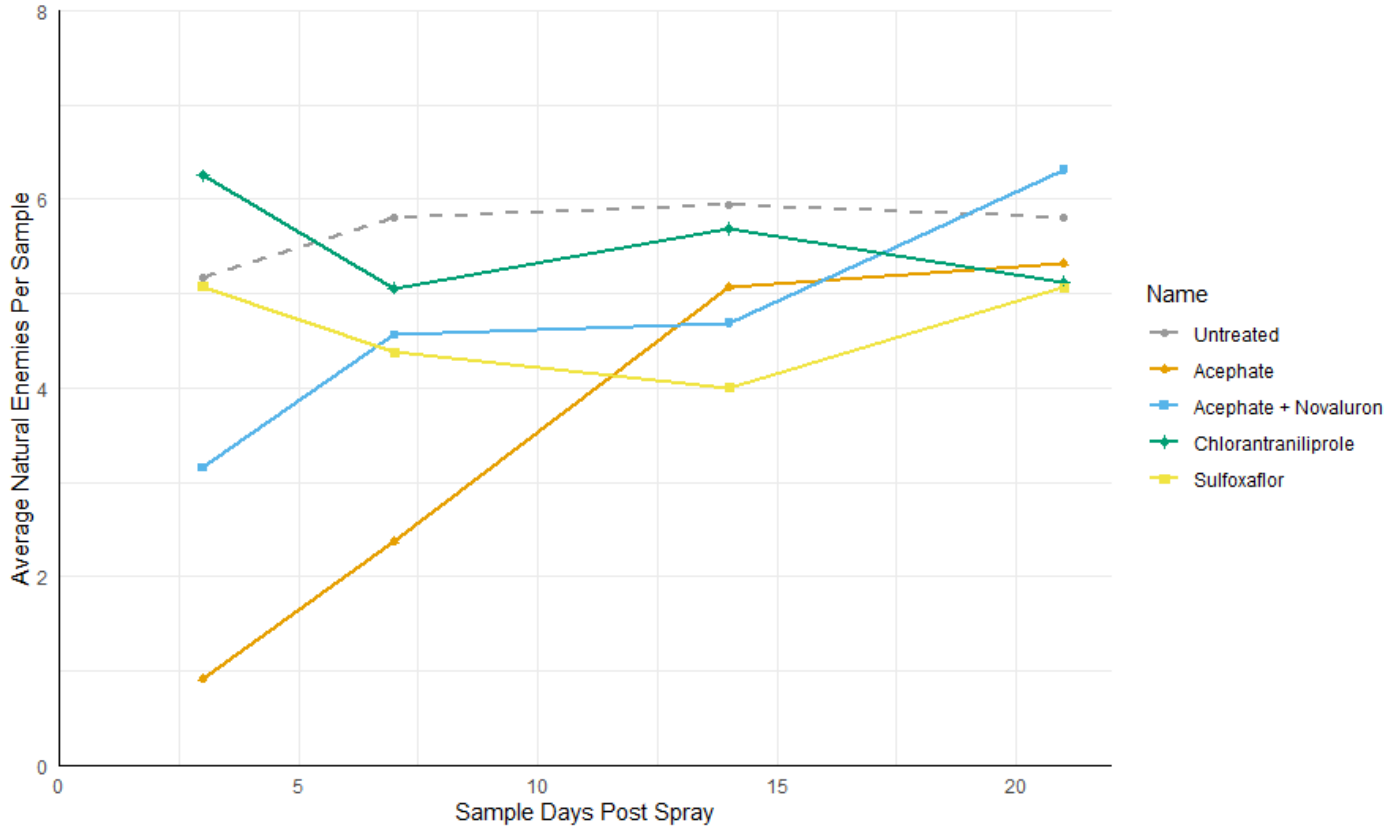
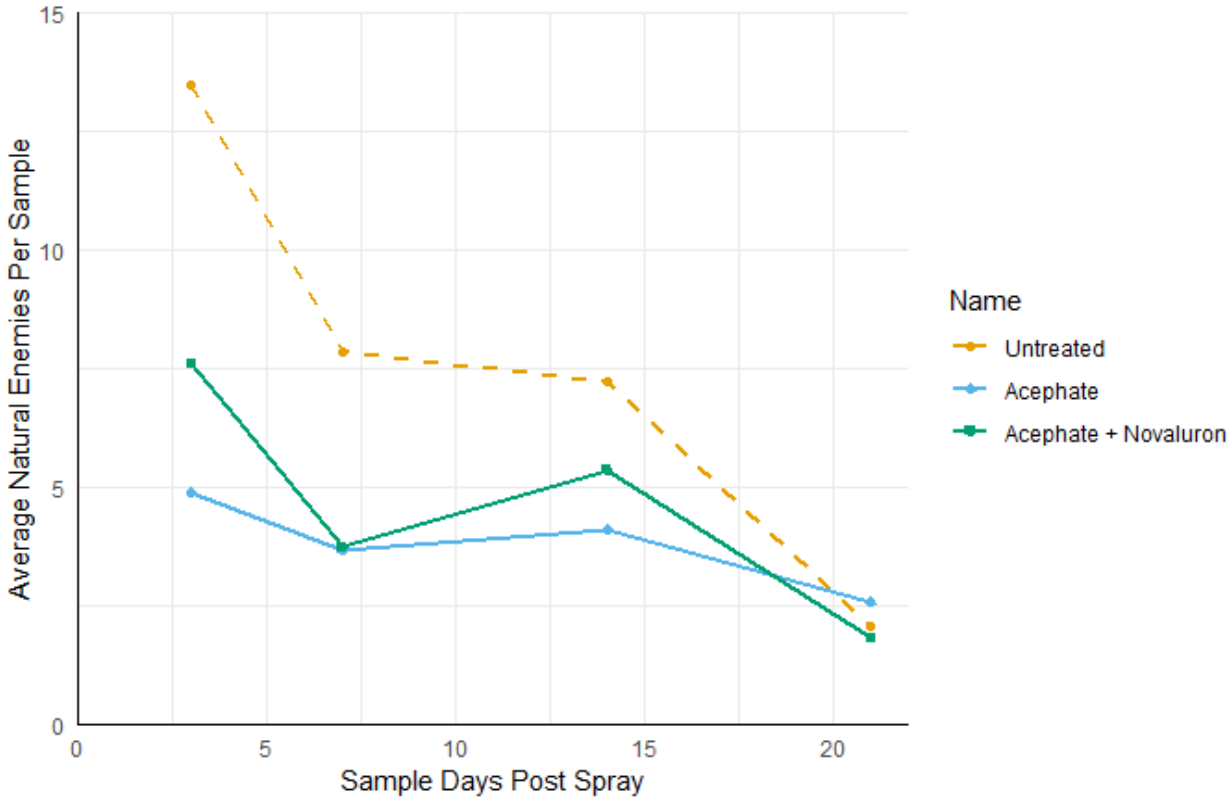


Figure 3.5 Average natural enemies caught by treatment over time in the 2021 Acephate Toxicity Study.



Supplemental Information

Table S3.1 Study location and application information.

State	City	Study	Latitude	Longitude	Year	Sprayer Model	Nozzle Type	Liters per hectare	Sprayer Width in Rows
Alabama	Prattville	Acephate Toxicity	32.4269	-86.4460	2021	Ground Sprayer	-	-	-
North Carolina	Clayton	RR Screening	35.6653	-78.5065	2020	Backpack sprayer	TeeJet XR8002VS		2
		RR Screening	35.6683	-78.5122	2021	JD 6000 Sprayer	#3 Orifice	476.8	8
	Goldsboro	RR Screening	35.3659	-78.0479	2020	Backpack sprayer	TeeJet XR8002VS		2
		RR Screening	35.3967	-78.0378	2021	JD 6000 Hi/Cycle Sprayer	Tee Jet Twin Jet TJ60-8003EVS	140.3	16
	Jackson Springs	Acephate Toxicity	35.1945	-79.6896	2021	Decloet DS 400 HighBoy Sprayer	TeeJet XR #4	140.3	8
	Plymouth	RR Screening	35.8489	-76.6572	2020	JD 6000 Sprayer	TeeJet TX-12	112.3	4
		RR Screening	35.8519	-76.6569	2021	JD 6000 Sprayer	TeeJet TX-12	112.3	4
South Carolina	Blackville	Acephate Toxicity	33.3645	-81.3296	2021	-	-		
Virginia	Suffolk	RR Screening	36.6839	-76.7655	2020	Lee Spider Sprayer	Xr11002-VS	186.0	4
		RR Screening	36.6632	-76.7310	2021	Lee Spider Sprayer	Xr11002-VS	186.0	4