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Evaluation of Thresholds, Control, and Behavioral Responses of Tobacco Thrips, Frankliniella fusca (Hitch), and Tarnished Plant Bugs, Lygus lineolaris (Beauvoris), in ThryvOn Cotton

Adam Young Whitfield
University of Arkansas-Fayetteville

Evaluation of Thresholds, Control, and Behavioral Responses of Tobacco Thrips, *Frankliniella fusca* (Hitch), and Tarnished Plant Bugs, *Lygus lineolaris* (Beauvoris), in ThryvOn Cotton

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Benjamin C. Thrash, PhD.
Thesis Director

Nicholas R. Bateman, PhD.
Co-Committee Chair

Gustav M. Lorenz III, PhD.
Committee Chair

Bill Robertson, PhD.
Committee Member (ex-officio)

Abstract

Tobacco thrips are an important pest in Mid-South cotton production. Thrips are a pest of seedling cotton, feeding on the leaf tissue of plants which can result in stunted growth, delayed fruiting, loss of apical dominance, and possible stand loss. Field studies were conducted in 2021 and 2022 to evaluate Thryvon, a new transgenic trait in cotton that produces the *Bt* toxin Cry51Aa, for control of tobacco thrips. Thryvon cotton was tested at three locations in Arkansas: Marianna, Tillar, and Keiser. These studies evaluated thrips control on ThryvOn vs non-ThryvOn cotton and the effect of insecticide seed treatments on ThryvOn cotton. Untreated ThryvOn cotton had fewer thrips and less injury than untreated non-ThryvOn cotton. ThryvOn cotton in combination with an insecticide seed treatment did not increase thrips control compared to untreated ThryvOn cotton. Controlled environment no choice tests were completed in 2021 to determine the feeding preference of adult tobacco thrips when presented a choice between ThryvOn and non-ThryvOn cotton seedlings. A higher number of thrips were observed on non-ThryvOn cotton seedlings. Results from these studies indicate that ThryvOn has the potential to be a valuable tool for thrips management.

Tarnished plant bug (TPB) is the most economically important pest in mid-south cotton production causing square loss, deformed flowers, and damaged bolls, which ultimately reduces yield. TPB is difficult to control with growers averaging 4-6 insecticide applications per year. A field study was conducted at one location in 2021 and two locations in 2022 to evaluate ThryvOn for TPB control and the potential it has to change TPB management. These studies compared ThryvOn and non-ThryvOn cotton that was either untreated or sprayed at 1x, 2x, or 3x the current 3 TPB per 1.5 row m threshold. Based on our current 3 TPB per 1.5 row m threshold, ThryvOn required 2 applications for TPB compared to 5 in non-ThryvOn at Marianna. Untreated

ThryvOn cotton had 14.8% higher square retention when compared to untreated non-ThryvOn. Yields in unsprayed ThryvOn were no different than any of the sprayed ThryvOn treatments. Behavioral response tests in the field for TPB were conducted with a cage study in 2022. Adult TPB were caged on the upper 4 nodes of non-ThryvOn and ThryvOn cotton. A greater TPB mortality and square retention was observed on ThryvOn cotton when compared to non-ThryvOn cotton. Results from these studies indicate that ThryvOn will be a valuable tool in TPB management.

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Chapter I – Introduction

Cotton Production

Cotton, *Gossypium hirsutum* L, is the foremost commercially important fiber crop in the world and is deemed as the backbone of the textile industry (Chakravarthy et al. 2014). As the world's leading natural fiber and the second largest oilseed crop, cotton is a main stay of U.S. and global economies. In the U.S. alone, cotton is harvested on approximately 4.8 to 6.5 million hectares on a yearly basis and is valued in excess of $38 billion dollars (Wilkins, Rajasekaran and Anderson 2000).

The genus *Gossypium* L. includes four species of cultivated cottons broken up into two groups. American tetraploid species (*G. barbadense and G. hirsutum*) dominate worldwide cotton production when compared to Old World diploid cultivars (*G. arboretum* and *G. herbaceum*). *Gossypium hirsutum*, upland cotton, dominates the cotton production market (90% of world production). Upland cotton is native to Central America, Mexico, the Caribbean and southern Florida (Tyagi et al. 2014).

During domestication, day-neutral stocks were selected which allowed a market for cotton to be grown in the United States. Cotton cultivation in the U.S. dates back to the early seventeenth century (Smith et al. 1999). One of the most important events in U.S. cotton breeding history was the introduction of Mexican highland stocks in the early 1,800s, which contributed to the foundation of current upland germplasm (Wendel et al. 1992).

Upland cultivars currently are grown in more than 40 nations in both tropical and temperate latitudes, from 47° N in the Ukraine and 37° N in the United States to 32° S in South America and Australia (Wendel, Brubaker and Percival 1992). The cotton growing area in the US can be broadly divided into four regions: Western, Southwestern plains, Mid-South (the delta), and Southeast (Tyagi et al. 2014).

Cotton Biology

Cotton is a perennial shrub, grown as an annual. It has an indeterminate growth habitat and a very dynamic growth response to environment and management. The average number of days required for planting to harvest ready in the midsouth is 130 to 160 days (Oosterhuis 1990). Cotton seed germination and seedling emergence are the first stages of cotton growth. Environmental factors such as temperature, water availability, and soil conditions like compaction, rhizosphere gases, seed and seedling pathogens, and the interactions among these and other biotic and abiotic factors, directly affect the time it takes for cotton seedlings to emerge post planting (Stewart et al. 2010). Cotton seedling emergence normally takes place 4 to 14 days after planting. At the soil surface, the hypocotyl straightens and pulls the folded cotyledons out of the soil, a process known as epigeal germination. After the cotyledons are pulled through the soil surface, they unfold and expose the epicotyl and the apical meristem, or growing point, which will be the source of subsequent growth (Ritchie et al. 2007). The cotyledons serve a dual role in germination. Before they unfold, they supply stored food to the germinating seedling. After the cotyledons unfold, they produce chlorophyll, become green, and produce energy through photosynthesis. The apical meristem emerges at the base of the cotyledons, and all further vegetative and reproductive growth of the plant occurs through the meristems (Ritchie et al. 2007).

The main stem of the plant has an indeterminate growing point that produces leaves in a three-eighths spiral. Two buds are formed at the base of each leaf. If the axillary bud develops it produces a vegetative branch. If the extra-axillary bud, which may be located either to the right or left of the axillary, develops it produces a fruiting branch. The vegetative branches are structurally like the main stem and may replace the main stem. In addition to arising from the main stem of the plant, vegetative branches may develop from any of the axillary buds of either the vegetative or the fruiting branches (Eaton 1955).

Branches on a cotton plant can be classified as either vegetative branches (monopodia) or fruiting branches (sympodia) (Bednarz et al. 2007). New fruiting branches develop approximately every 3 days and new squares are produced at new positions on a fruiting branch approximately every 6 days. During the 21-day period from square to bloom, there are several recognized developmental stages of the cotton flower bud. A "pinhead" square is the first stage at which the square can be identified. The next stage of square growth is "match-head". After that a "one-third grown" or "screw head" square can be observed. Just prior to the time the flower opens, a candle shape can be seen. This period of square development prior to bloom is called "squaring".

Once the cotton begins to bloom, it is said to be "flowering". A cotton plant typically blooms or flowers for about 6 weeks (Wright et al. 2011). Thus, until the cotton begins to produce fruit, the stage of development is discussed in terms of leaves or nodes. Flowering is important to cotton production because pollinated flowers form cotton bolls. The bloom process takes several days, and bloom age can be estimated by the bloom characteristics. On the first day the flower will be white, changing to a pink-like color the second day, and a red color on the third day. Approximately 5 to 7 days after a flower appears it usually dries and falls from the plant exposing the developing boll (Oosterhuis 1990). During the flowering period, the stage of cotton development can also be discussed in terms of the number of nodes above the uppermost white flower (NAWF). As flowering approaches, the top of the plant and eventually ceases, the plant will put all its energy into boll development. A cotton plant reaches "cutout", which is when a cotton plant reaches 4 or 5 NAWF (Wright et al. 2011).

Cotton bolls develop rapidly after fertilization and reaches its full size within three weeks. An additional four to five weeks are required for boll maturation (Wright et al. 2011). During the maturation phase, boll development proceeds with the formation of seeds and lint, leading up to boll opening, defoliation, and harvesting (Oosterhuis 1990). Seeds attain their full size about three weeks after fertilization, but do not reach maturity until shortly before the boll opens (Wright et al. 2011).

Thrips Biology

Thrips fall in the order Thysanoptera can be divided into two suborders- Tublifera and Terebrantia. The Tublifera species oviposit eggs on the plant tissue surface while Terebrantia species insert eggs within the plant tissue. The suborder Terebrantia contains all the species of thrips considered to be pests on cotton in the U.S. The most economically important genus of thrips to cotton is *Frankliniella* which contains the tobacco thrips species (Graham et al. 2019).

Thrips are usually the first insects to attack cotton, causing significant damage and economic losses. Thrips are the most important pest of seedling cotton in Arkansas and the Mid-South. One hundred percent of cotton acres in Arkansas are infested with Thrips (Cook 2019). Five thrips species commonly infest cotton seedlings in the United States. These include western flower thrips; flower thrips, *Frankliniella tritici* (Fitch); soybean thrips, *Neohydatothrips variabilis* (Beach); onion thrips, *Thrips tabaci* (Lindeman); and tobacco thrips, *Frankliniella fusca* (Hinds). Tobacco thrips are widely distributed across the Mid-South and southeast cotton production regions and are the predominate species in many areas (Cook et al. 2011).

Tobacco thrips in Arkansas comprise up to 84% of all thrips species found on seedling cotton (Clarkson 2014). Adults are typically 1 to 2 mm long, with or without wings and depending on the species the color can vary from yellowish to black. Adults have two pairs of wings that are held folded behind the back except during flight. Winged thrips have four long narrow wings with few or no veins. A fringe of hairs on the posterior margin characterizes each wing but they can only be seen under a microscope. Thrips can fly but they are typically windblown from field to field. Immatures do not have developed wings, they're smaller in size and typically are lighter in color (Graham and Stewart 2018). Under optimal conditions a single female thrips may lay up to 100 eggs during her lifetime (Layton and Reed et al. 2002). Eggs hatch after 2-3 days and larvae development lasts 2-13 days. The prepupa drops to the soil after 1-5 days, pupates, and adults emerge from the soil after 1-10 days (Hinds 1903, MaGill 1927, Bailey 1938, Lublinkhof and Foster 1977, Lowery et al. 1992). Thrips can be found on numerous crop and weed species, many of which are found within cotton production environment (Cook et al. 2011).

Thrips Feeding Injury and Sampling

Thrips use their rasping sucking mouthparts to feed on young meristems. The damaged cells die, but the cells around them continue to expand and divide, resulting in ragged and crinkled leaves, silver or whitish appearance, reduction in size of the first true leaf, and in some cases plant death (Ritchie et al. 2007). The first symptoms of damage are small areas of feeding on the cotyledonary leaves, which soon appear silver or whitish. Immatures and adults show preference for the small leaves and stipules in the bud, resulting in ragged and crinkled leaves as they expand and mature. Size of the first few true leaves is often greatly reduced. If feeding damage is severe enough to kill buds in the terminal, apical dominance is lost, and plants become excessively branched or distorted in appearance as secondary terminals form in leaf axils. Other problems related to thrips damage are increased seedling mortality, reduced plant height , reduced leaf area, delayed crop maturity, and yield loss (Burris et al. 1989). If infestation takes place under conditions such as cool weather and slower growth of seedlings then problems like stunting, delayed fruiting, loss of apical dominance and possible loss of stand are more likely to occur (Clarkson 2014).

Current Thrips Control Methods and Resistance

Control of thrips on cotton seedlings is generally achieved with use of prophylactic at-planting insecticide treatments. This practice has been widely adopted because damage from thrips can occur quickly after seedling emergence. In the past insecticides such as aldicarb (AgLogic 15G®; AgLogic Chemical Company LLC; Chapel Hill, NC) were the primary control method, applied as an in-furrow granular. Aldicarb was previously on the market as Temik 15G® (Bayer Crop Science; St Louis, MO) for 40 years, and was quite effective, but it was discontinued in 2010. Aldicarb was continued under the name AgLogic 15G in 2015, but between 2010 and 2015 growers had switched to neonicotinoid seed treatments for thrips control (Attaway 2016). Neonicotinoid seed treatments had many benefits over in-furrow applications of aldicarb including, a cheaper and more reliable method of application, convenience to the grower, reduced toxicity to growers, and reduced equipment costs (Taylor and Harmon 1990). Due to these benefits, coupled with aldicarb supply issues and the fact that many growers no longer have the equipment to apply granular in-furrow insecticides, growers are hesitant to switch back to in-furrow applications.

Insecticide seed treatments are a very common approach when it comes to a proactive thrips management strategy. Seed treatments such as acephate (Orthene 97®; Valent USA; Walnut Creek, CA) , imidacloprid (Admire Pro®; Bayer Crop Science; St Louis, MO), and thiamethoxam (Centric 40WG®; Syngenta Crop Protection Inc; Greensboro, NC) are used on all cotton acres in the Mid-South (Cook et al. 2011). Residual activity of at-planting insecticides ranges from 2 to 4 weeks after planting.

Neonicotinoids, are the primary seed treatments used by cotton growers (Cook et al. 2011). Prior to 2011, producers were generally able to obtain adequate control of tobacco thrips by using either an in-furrow application or a seed treatment (Greene 2010). Starting in 2011, a decline in neonicotinoid efficacy against tobacco thrips was observed throughout the Mid-South region (Catchot et al. 2013, Lorenz 2013, Stewart 2013). As poor efficacy became more common, neonicotinoid seed treatments are generally supplemented with foliar-applied insecticides to control heavy infestations and are currently the principal method for managing tobacco thrips. Currently, researchers have established an action threshold of 2-5 thrips per plant with damage present for thrips management. The widespread occurrence of neonicotinoid resistance in tobacco thrips throughout the south-eastern cotton production region has led to increased reliance on supplemental foliar and in-furrow applications of systemic insecticides for thrips control (Chappell et al. 2020).

As of 2007, neonicotinoids made up over half of the global insecticide market and represented approximately $535 million in sales (Elbert et al. 2008). Resistance in tobacco thrips was not reported for any insecticides until recently (Huseth et al. 2016). Huseth et al. (2016) reported tobacco thrips resistance to the neonicotinoid class, namely, imidacloprid, and thiamethoxam, in more than half of their assays drawn from field populations in cotton production areas of the southern United States. Krob et al. (2022) conducted field trials and bioassays from 2018 to 2021 evaluating resistance of tobacco thrips to insecticides in the southern United States. Bioassay results suggest that tobacco thrips have developed resistance to acephate and other organophosphate insecticides; however, this resistance seems to be most severe in Arkansas, Tennessee, and the Delta region of Mississippi. However, it is evident that many populations of tobacco thrips are resistant to multiple classes of insecticides. Further research is needed to determine heritability and resistance mechanisms. Because of tobacco thrips resistance, Mid-South cotton producers are seeking alternative methods of control.

Tarnished Plant Bug Biology

The tarnished plant bug (TPB), *Lygus lineolaris* (Beauvois), is a true bug in the Miridae family that feeds with piercing sucking mouthparts (George et al. 2021). TPB feeds primarily on the meristematic tissues of cotton squares and small bolls, resulting in square abscission, damage to the anthers and the staminal column, and death of pinhead squares (George et al. 2021). TPB adults are greenish to brownish in color with reddish-brown markings. Adults are 5–6 mm long and 2.5–3 mm wide with a flattened body that is oval in outline. Adults have a characteristic light-colored “V” behind the head and two light-colored patches further back on the wings (George et al. 2021).

The TPB has three distinct life stages: egg, nymph, and adult. The life cycle begins as an adult overwintering in leaf trash (Cleveland 1982). The TPB life cycle averages 22 to 46 days depending on temperature (Fleischer and Gaylor 1988, Snodgrass et al. 1984). A female TPB can lay up to 175 eggs over the course of their life cycle at an average temperature of 27 degrees C (Ugine 2012). Approximately 5 days are required to complete the first instar stage, with approximately 3 days required to complete each of the second, third, and fourth instar stages, with the fifth instar stage taking approximately 5 days to complete (Ridgeway and Gyrisco 1960).

The presence of flowering vegetation is key to TPB attraction and reproduction, as flowers are the preferred feeding site. TPB have a broad host range, with over 385 documented host plants (Young 1986). Host plants range from wild plants and weeds to fruits, vegetables, and agronomic crops. The most important non-crop wild hosts of TPB populations in the mid-southern United States are tall goldenrod (*Solidago altissma* L.), pigweed (*Amaranthus* spp.), pinkweed (*Polygonum penslyvanicum* L.), white heath aster (*Aster pilosus*),and daisy fleabane (*Erigeron* spp.) (George et al. 2021). TPB has been documented to host on many species of weeds in field margins and then migrate into cotton fields once the weedy hosts senesce or are destroyed.

Tarnished Plant Bug Feeding Injury and Sampling

TPB is a highly polyphagous mirid that damages many economically important plants across much of the Americas and is found in all the agricultural regions of the United States. TPB feeds on cotton squares and small bolls, which reduces lint quality and yield and causes millions of dollars in damage annually (George et al. 2021). Musser et al. (2009) reports a trial across 19 locations throughout the Mid-South during 2006 and 2007. Threshold treatments ranged from a weekly automatic insecticide application to a very high threshold of 10 TPB per 1.5 row-m on a black drop cloth. Results from this study concluded that an economic threshold should be between 1.6 and 2.6 TPB per 1.5 row m during the flowering period. Arkansas's current action threshold, recommended by the University of Arkansas 2023 MP 144, is 3 TPB per 1.5 row m or 8-12 TPB per 100 sweeps from early square through cutout (NAWF=5). Weekly NAWF measurements begin at approximately first flower and continued once per week until NAWF equals 5. Once a field reaches cutout, heat unit accumulation was initiated using the following equation: [(Daily high temp. + Daily low temp.)/2] - 60°F where 60°F is the lower threshold for growth (Benson et al. 1999). The University of Arkansas 2021 Cotton Quick Facts recommends terminating TPB management at 250 heat units beyond cutout (NAWF=5). After cutout treat for 6 TPB per 1.5 row m until termination.

The TPB has piercing and sucking mouthparts which pierce the host tissue. Food is then taken up by sucking action. Swellings or lesions appear externally in 2 or 3 days at feeding puncture sites on cotton stems or petioles (King and Cook 1932). Flemion et al. (1952) found that TPB deposit saliva in the host tissue during feeding. Flemion et al. (1954) reported that tissue breakdown after TPB feeding resulted from the action of the stylets during the feeding process. The very small, sharp stylets are rapidly thrust into and through many cells. The cell walls of the host tissue, the contents of the cells, along with some of the injected saliva, are taken up by the insect. Typically, most economic damage from TPB in cotton occurs from the third week of squaring through the fourth week of bloom (George et al. 2021). Fruit retention during the pre-bloom period is critical to maximize yield.

Current Tarnished Plant Bug Control Methods and Resistance

Chemical insecticides are currently the most widely used method of control for TPB in cotton in the Mid-South. Several classes of insecticides are needed to control this pest. TPB is difficult to control with growers averaging 4-6 insecticide applications per year (Cook 2019). Insecticide options currently available include organophosphate, carbamate, neonicotinoid, pyridinecarboxamide, pyrethroid, insect growth regulators, and sulfoxamine classes (Snodgrass et al. 2009). Multiple insecticide applications are very expensive for producers, they are continually seeking alternative methods of control. It is currently recommended that growers' budget approximately $247 per hectare to allow for proper control of TPB throughout the season (Watkins 2019). Fields should be scouted twice a week to ensure populations are controlled as soon as threshold is reached (Gore et al. 2007). Heavy reliance on insecticides has led to a decline in the efficacy of most insecticides against TPB. There is confirmed TPB resistance to organophosphates, carbamates, pyrethroids, and neonicotinoids (Snodgrass et al. 2009). A multitactical approach is required to obtain economical control. Early planting dates (Bouquet and Clawson, 2009) combined with an early maturing cotton variety (Adams et al. 2013) can significantly reduce the number of insecticide applications needed to control TPB throughout the growing season. Reducing plant height and opening the plant canopy with plant growth regulators, such as mepiquat chloride (Pix Plus®; BASF; Research Triangle Park, NC), can potentially increase the effectiveness of insecticides needed late in the growing season (Graham 1985). Applying selective herbicides to areas such as turn rows, ditches, and roadsides for control of early season hosts can effectively help to reduce the cost of control later in the growing season (Snodgrass 2003, Snodgrass et al. 2006, Gore et al. 2010).

Bacillus thuringiensis (*Bt*) has been known to have insecticidal properties for

over a century. The utility of *Bt* toxins increased substantially beginning in 1996, when the first transgenic crops which expressed *Bt* toxins became commercially available (Perlak et al. 13 2001). The widespread adoption of *Bt* cotton caused a shift in the key insect pest of cotton from the heliothine complex to TPB (Musser et al. 2009). *Bt* cotton, combined with the eradication of the boll weevil, *Anthonomus grandis* (Boheman), has caused TPB to go from a secondary pest to a primary pest, because applications targeted at these pests, which provided management of TPB, are no longer required (Musser et al. 2009).

Development of Cry51Aa (ThryvOn)

Bacillus thuringiensis (*Bt*) was first isolated by a Japanese scientist in 1901 (Ishiwata 1901). The utility of *Bt* toxins increased substantially beginning in 1996, when the first transgenic crops to express *Bt* toxins became commercially available (Perlak et al. 13 2001).

These varieties provided excellent control of the heliothine complex. The heliothine complex consists of two caterpillars that feed on fruiting structures of cotton, the tobacco budworm, *Heliothis virescens* (Fabricius), and bollworm, *Helicoverpa zea* (Boddie) (Siebert et al. 2008). The widespread adoption of *Bt* cotton caused a shift in the key insect pest of cotton from the heliothine complex to the TPB (Musser et al. 2009). While many *Bt* proteins have shown excellent insecticidal activity against lepidopteran, coleopteran, and dipteran insects (Schnepf et al. 1998, Van Frankenhuyzen 2009), few have shown adequate activity against hemipteran insects (Walters and English 1995, Porcar et al. 2009).

Today the natural fiber industry, especially cotton, must use newer technologies, such as genetic engineering, to increase yield and quality (John 1997). Rising production costs and stagnant pricing are the factors most frequently cited as posing a threat to cotton production in the U.S. (Wilkins et al. 2000). Genetic engineering has enabled the incorporation of potential candidate genes for several beneficial traits thereby surpassing the limitations normally associated with the conventional methods of crop improvement (Chakravarthy et al. 2014).

Genetic engineering offers a directed method of plant breeding that selectively targets one or a few traits for introduction into the crop plant. The development and commercial release of transgenic cotton plants that have been genetically modified relies exclusively on two basic requirements. The first being the ability to “transform” a plant by introducing a gene, or genes, into the cotton genome that are stably transmitted and expressed in the progeny of subsequent generations. The second requirement is the need to regenerate fertile plants derived from individual cells, and this has proven to be the much more difficult of the two requirements to surmount (Wilkins et al. 2000).

One well-established method for DNA transfer into cotton is by Agrobacterium tumefaciens-mediated transformation (John 1997). The mechanism of gene transfer between Agrobacterium and the plant kingdom has facilitated the insertion of beneficial alien genes into diverse plant genomes (Barton et al. 1983; John 1997). Agrobacterium-mediated transformation has been the most widely used and preferred method of transferring genes into plants (Wilkins et al. 2000).

Baum et al. (2012) reported a *Bt* protein with insecticidal activity against both *Lygus hesperus* (Knight) and *Lygus lineolaris* (Beauvois). In addition to insecticidal activity against mirids, this protein also has activity against at least some thrips (Bachman et al. 2017). The mode of action for Cry51Aa has not yet been identified but the *Bt* protein Cry51Aa is the first cotton biotech trait that will provide season-long protection against TPB and thrips species and may help reduce the need for some insecticide applications. Thryvon technology (Cry51Aa) offers protection against TPB and thrips.

Graham (2018) reported that ThryvOn provides as good or better protection from thrips injury compared to the current best alternative strategy (IST + foliar application). There was significantly higher square retention, fewer TPB adults prior to bloom, and fewer total TPB nymphs, especially large nymphs, during bloom in ThryvOn plots compared to non-ThryvOn plots. When plots were managed for TPB using current thresholds, no difference in yield was observed, however an average of 1.2 fewer insecticide applications were required for ThryvOn plots over non-ThryvOn. With growers seeking alternative methods of control that provide season long protection, ThryvOn technology must be evaluated to determine if it can be incorporated into future cotton management strategies.

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Chapter II - Field evaluation of ThryvOn Technology for Control of Thrips (Thysanoptera: Thripidae) and Tarnished Plant Bugs (Hemiptera: Miridae) in Cotton

Abstract

Tobacco thrips, *Frankliniella fusca* (Hinds), and tarnished plant bugs (TPB), *Lygus lineolaris* (Beauvois), are two of the most economically important pests in Mid-South cotton production. Field experiments were conducted in 2021 and 2022 in Arkansas to evaluate the effects of ThryvOn technology on tobacco thrips and TPB in cotton. Tobacco thrips is one of the most important pests in Mid-South cotton production. Thrips are a pest of seedling cotton feeding on the leaf tissue of plants which can result in stunted growth, delayed fruiting, loss of apical dominance, and possible stand loss. TPB is the most important pest in Mid-South cotton production causing square loss, deformed flowers, and damaged bolls, ultimately reducing yield. TPB is difficult to control with growers averaging 4-6 insecticide applications per year. ThryvOn cotton was evaluated at three locations in Arkansas across the two years: Marianna, Tillar, and Keiser, AR. The tobacco thrips studies evaluated efficacy and feeding injury in ThryvOn and non-ThryvOn cotton. After sampling, ThryvOn cotton had fewer total thrips (75%) and less feeding injury (68%) compared to non-ThryvOn cotton. Results from this study indicate that ThryvOn has the potential to be a valuable tool for controlling thrips. The TPB studies consisted of ThryvOn and non-ThryvOn cotton that was either untreated, or sprayed at 1x, 2x, or 3x the current 3 TPB per 1.5 row m threshold. Based on the 3 TPB per 1.5 row m threshold, ThryvOn cotton required less insecticide applications for TPB control compared to non-ThryvOn cotton. Yields in unsprayed ThryvOn were no different than any of the sprayed ThryvOn treatments. Results from this research indicate that ThryvOn technology will be a valuable addition to an overall insect management program in cotton.

Introduction

Tobacco thrips are the most important pest of seedling cotton in Arkansas. One hundred percent of cotton acres in Arkansas are infested with thrips (Cook 2019). Five thrips species commonly infest cotton seedlings in the United States. These include western flower thrips; flower thrips, *Frankliniella tritici* (Fitch); soybean thrips, *Neohydatothrips variabilis* (Beach); onion thrips, *Thrips tabaci* (Lindeman); and tobacco thrips, *Frankliniella fusca* (Hinds) (Cook et al. 2011). Thrips injure cotton by feeding in the terminal area of the plant. This terminal feeding disrupts normal growth of the plant leaf structure. This can result in severely deformed leaves, aborted terminals, and greatly reduced leaf area. This injury reduces the photosynthetic capacity of the plant resulting in lower plant vigor, stunting, increased susceptibility to plant diseases, and often a delay in maturity. If not controlled, thrips injury can severely reduce stands (Studebaker et al. 2002). Because thrips are early-season, persistent pests, most growers use a proactive approach to control them, by using at-planting insecticides which can be applied as a seed treatment or in-furrow (liquid or granular) treatment (Krob et al. 2022). At-planting, insecticide treatments may fail to provide adequate thrips control because of insecticide resistance, severe thrips pressure, or unfavorable growing conditions; so foliar-applied insecticide applications may be needed for supplemental control (Cook et al. 2020). Widespread adoption of a treatment program that increases neonicotinoid use by up to ninefold through the use of neonicotinoid seed treatment in combination with an in-furrow application will not only increase selection for resistance but could also negatively impact water quality and ecosystem health (Huseth et al. 2016). Control of thrips in cotton costs growers on average $16.01 per hectare (Williams 2015). Documented control issues in the Mid-South (Darnell et al. 2015, 2016) and the southeast (Huseth et al. 2016) have led to the need for alternative methods of control.

TPB is the most important pest in Mid-South cotton production causing square loss, deformed flowers, and damaged bolls, leading to yield losses. TPB is a true bug in the Miridae family that feeds with piercing sucking mouthparts (George et al. 2021). TPB feeds primarily on the meristematic tissues of cotton squares and small bolls, resulting in square abscission, damage to the anthers and the staminal column, and death of pinhead squares (George et al. 2021). TPB is difficult to control with growers averaging 4-6 insecticide applications per year in Arkansas. Fields should be scouted twice a week to ensure populations are controlled as soon as threshold is reached (Gore et al. 2007). Heavy reliance on insecticides for management has led to a decline in the efficacy of TPB for some insecticides. Control of TPB has become increasingly difficult, leading to the need for alternative means of control.

Bollgard® cotton (Monsanto Company; St. Louis, MO) was initially available in the USA in 1996, and almost 725,000 hectares were planted that year (Perlak et al. 2001). Although no commercial cotton varieties expressing *Bt* toxins for control of hemipteran or thysanopteran pests have been commercially available prior to 2023, Bayer Crop Science (Monsanto Company; St. Louis, MO) has developed a new *Bt* protein, Cry51Aa2.834_16 (ThryvOn technology) with activity against thrips and TPB (Baum et al. 2012, Gowda et al. 2016, Bachman et al. 2017, Graham and Stewart 2018). The objectives of this study were to evaluate ThryvOn for control of thrips and TPB and if it will allow for a reduction in insecticide applications needed for the management of both pests. Additionally, TPB thresholds were reevaluated on ThryvOn cotton to determine if thresholds were similar to non-ThryvOn cotton or if they could be increased.

Materials and Methods

Plot Establishment

Experiments were conducted in 2021 and 2022 at the Lon Mann Cotton Research Station located in Marianna, AR (Marianna), on a grower's field in Tillar, AR (Tillar), and the Northeast Research and Extension Center in Keiser, AR (Keiser). All cotton seed was treated by the manufacturer prior to planting with Acceleron Standard (Bayer Crop Science; St. Louis, MO). Plots at all locations were fertilized and managed for weeds according to University of Arkansas Extension recommendations. Trials in 2021 were planted on 20 May at Marianna and 2 June at Tillar. Trials in 2022 were planted on 11, 18, and 20 May at Marianna, Tillar, and Keiser, respectively. The cotton varieties planted were ThryvOn (DP 2131 B3TXF; Bayer Crop Science, St Louis, MO) and non-ThryvOn (DP 2055 B3XF; Bayer Crop Science; St Louis, MO). Plot size was 4 rows (0.97 m centers) by 15 m, with a seeding rate of 13 seed per m of row. The experiment was arranged in a randomized complete block design with four replications per treatment.

Thrips Observations

In 2021 and 2022, across all three locations, thrips observations were made within ThryvOn and non-ThryvOn cotton. Samples were taken at random positions throughout the established ThryvOn and non-ThryvOn plots to compare thrips densities between the technologies. In 2021, additional thrips observations were made at Marianna. Plots were planted on 7 Jul to maximize exposure to thrips. Plot size was 8 rows (0.97 m centers) by 91 m, with a seeding rate of 13 seed per m of row was used. Treatments were ThryvOn and non-ThryvOn cotton in combination with a fungicide only untreated check or a fungicide + Imidacloprid (Gaucho®; Bayer Crop Science; St. Louis, MO) insecticide seed treatment for a total of four treatments.

Thrips Sampling

Cotton was sampled at the 1-2 and 3-4 leaf stage to estimate thrips density. Four random samples were taken from the two centers rows of a plot in both ThryvOn and non-ThryvOn, (8 total samples) with each sample consisting of five plants. Plants were cut at the ground level and placed in 25.2 cm x 17.8 cm x 8.6 cm jars containing 70% ethyl alcohol. Each plant was removed from the jar and the jar was then rinsed with 70% ethyl alcohol over a filter paper placed on top of a glass jar to collect any thrips. The filter paper was then rinsed with 70% ethyl alcohol into a gridded 100 mm x 15 mm petri dish and the thrips counted underneath a dissecting scope. Thrips were counted and categorized as either adult or immature.

In 2022, whole-plot visual ratings of thrips injury were taken at the 1-2 and 3-4 leaf stage. Thrips ratings were on a 0-5 scale, with 0 representing no injury to any plant in the plot and 5 representing no living plants in the plot.

Number of thrips and thrips injury ratings were analyzed to show the overall impact of each management strategy on those variables. All thrips data was used for analysis. Data was combined and analyzed by using JMP pro version 16.2.0 with a mixed model analysis of variance. This allowed for comparisons between individual treatments. Individual treatments were set as fixed effects. Year, location, and replication were set as random effects to allow inferences to be made over a range of environments. Means were separated using an all pairs, Tukey HSD analysis. Differences were considered significant at $\alpha=0.05$.

Tarnished Plant Bug Sampling

Beginning at the third week of squaring and through much of the blooming period, TPB densities were estimated using a black drop cloth (76 by 91 cm). Samples were taken by laying the cloth between the two center rows and vigorously shaking the plants over the black drop cloth from each row. Two samples were taken per plot, for a total of 3.0 m of row being sampled, to provide an accurate estimation of TPB within the plot. TPB nymphs and adults were recorded separately. TPB nymphs were visually separated based on size as either small (1st or 2nd instars) or large (3rd, 4th, and 5th instars). Small nymphs are about 1 mm long, yellowish green, and no wing pads are present. Large nymphs are yellow green to green, with yellow, green, or black spots, and wing pads are present on 5th instar. Square retention was monitored by examining the first position fruiting site on the third node down from the top on 25 plants in each plot, and the number of retained squares was recorded. A square was considered missing if it abscised when touched or the bracts were flared. Insecticide applications were made when treatment threshold was met. Treatment thresholds in 2021 included an untreated check, 6 nymphs per 3.0 row m (1x threshold), 6 large nymphs per 3.0 row m, 12 nymphs per 3.0 row m (2x threshold), 12 large nymphs per 3.0 row m, and 18 nymphs per 3.0 row m (3x threshold). Treatment thresholds in 2022 were adjusted to include an untreated check, 6 nymphs per 3.0 row m (1x threshold), 12 nymphs per 3.0 row m (2x threshold), 18 per 3.0 row m (3x threshold), 80% square retention, and 85% square retention. When a given threshold was met, plots were treated with sulfoxaflor (Transform WG®; Corteva Agriscience; Indianapolis, IN) at a rate of 61.3 g ai/ha. Applications were made using a Mud-Master sprayer fitted with 80-02 dual flat fan nozzles with 49.5 cm spacing. Spray volume was 93.5 L/ha at 276 kPa. Sampling was terminated when cotton reached five nodes above white flower plus 250 heat units (DD60s°F). Numbers of clouded plant bugs, *Neurocolpus nubilus* (Say), were also recorded at each sample date, as these pests could potentially be impacted by this technology and have influence on fruit injury and yield. Although specific thresholds have not been developed for clouded plant bugs, Stewart (2010) recommends counting each clouded plant bug as equivalent to 1.5 TPB.

Sample date was not included in data analysis because each treatment received insecticide applications a different number of times and at different timings during the season. Square retention and TPB numbers on drop cloths were analyzed to show the overall impact of each management strategy on those variables. TPB data from Marianna 2021, Marianna 2022, and Tillar 2022 were used for data analysis. Data was combined and analyzed by using JMP pro version 16.2.0 with a mixed model analysis of variance. This allowed for comparisons between individual treatments. Individual treatments were set as fixed effects. Year, location, and replication were set as random effects to allow inferences to be made over a range of environments. Means were separated using an all pairs, Tukey HSD analysis. Differences were considered significant at α=0.05. A linear regression analysis was conducted using JMP pro version 16.2.0 to compare percent square loss per nymph in untreated ThryvOn and non-ThryvOn across two years.

Yield

All plots were harvested at physiological maturity by harvesting rows two and three with a Case 1822 two row cotton picker equipped with HarvestMaster for small plot research. The HarvestMaster weigh system collects highly accurate measurements of plot weight. Seed cotton yield was recorded and adjusted to kg/ha.

Results

Thrips

Differences were observed between ThryvOn and non-ThryvOn for immature thrips (F=42.6; df=1,63; P <0.001), adult thrips (F=5.9; df=1,63; P=0.002), and total thrips (F=51.5; df=1,63; P<0.001). The number of immature thrips (25.1), adult thrips (10.1), and total thrips

(35.2) were reduced on ThryvOn compared to the number of immature thrips (126.3), adult thrips (19.5), and total thrips (145.4) on non-ThryvOn cotton (Fig. 1 A-C).

On a scale of 0-5 for thrips injury, with 0 representing no injury and 5 representing dead plants, differences were observed between ThryvOn and non-ThryvOn (F=43.9; df=1,39; P<0.001). A 3.5-fold reduction in thrips injury was observed on ThryvOn (0.6) compared to non-ThryvOn (2.1) cotton (Fig. 1. D).

A difference was observed between ThryvOn and non-ThryvOn, treated with an insecticide seed treatment, for immature thrips (F=38.5; df=3,15; P<0.001) and adult thrips (F=5.9; df=3,15; P=0.002). The greatest number immature (207.3) and adult (34.5) thrips were found on non-ThryvOn cotton that did not have an insecticide seed treatment (Fig. 2). The fewest number of immature (35.3) and adult (11.0) thrips were found on ThryvOn cotton treated with an insecticide seed treatment but there was no significant difference when compared to the number of immature (40.0) and adult (19.8) thrips found on ThryvOn cotton that did not have an insecticide seed treatment (Fig. 2).

Tarnished Plant Bug

For 2021 and 2022, not all treatments were initiated. Based on the current 3 TPB per 1.5 row m threshold, 1 to 5 insecticide applications were needed to manage TPB depending on the year and test location (Table 1 and 2). In 2021 at the Marianna location, 10 applications were required in the non-ThryvOn compared to 5 applications in the ThryvOn, across all treatment thresholds. In 2022 at the Marianna location, 16 applications were required in the non-ThryvOn compared to 8 applications in the ThryvOn, across all treatment thresholds. Overall, ThryvOn cotton required 50% less sprays across all treatments than non-ThryvOn cotton at two of the three locations. The Marianna location across two years, on average, required three less applications on ThryvOn cotton for control of TPB compared to non-ThryvOn cotton when sprayed at the 3 TPB per 1.5 row m threshold.

A difference was observed between ThryvOn and non-ThryvOn for small nymphs (F=5.5; df=1,137; P=0.002), large nymphs (F=21.4; df=1,137; P<0.001), and total TPB (F=10.7; df=1,137; P<0.001). Untreated ThryvOn reduced the number of TPB per 3.0 row m by 32.2% when compared to untreated non-ThryvOn. Fewer small nymphs (13.0), large nymphs (1.7), and total TPB (15.1) were found on ThryvOn cotton when compared to small nymphs (17.1), large nymphs (4.4), and total TPB (22.2) on non-ThryvOn cotton (Table 3). No difference was observed but a trend of fewer adult TPB were observed on ThryvOn cotton (0.4) when compared to non-ThryvOn cotton (0.7) (Table 3). A difference between ThryvOn and non-ThryvOn was observed for the number of small nymphs developing into large nymphs (F=10.0; df=1,137; P<0.001). A higher ratio of small to large nymphs were observed in the ThryvOn cotton (7.6) when compared to the non-ThryvOn cotton (5.7) (Table 3). A difference between ThryvOn and non-ThryvOn was observed for average % square retention (F=26.5; df=1,137; P<0.001). When comparing square retention across two years, untreated ThryvOn plots had greater square retention (86.7%) when compared to untreated non-ThryvOn plots (71.9%). On average, untreated ThryvOn cotton increased square retention by 14.8% when compared to untreated non-ThryvOn cotton (Table 3).

A linear regression analysis was conducted between % square loss and TPB nymphs in untreated ThryvOn (y= -0.27x + 90.61) and non-ThryvOn (y= -0.52x + 82.88) cotton across two years. ThryvOn technology reduced the % of square loss per nymph by nearly half when compared to non-ThryvOn. In non-ThryvOn cotton a 0.52% reduction in square retention per nymph was observed compared to a 0.27% reduction per nymph in ThryvOn cotton (F=24.1; df=2,137; P<0.001) (Fig. 3).

Yield

When comparing yields across two years, no treatment threshold in ThryvOn cotton provided a significant % yield increase when compared to the untreated ThryvOn. In the non-ThryvOn cotton, % yield increase was significant at the 1x (51%) and 2x (31%) threshold when compared to the untreated non-ThryvOn (F=3.6; df=1,4; P=0.008) (Fig. 4).

Discussion

More thrips were observed on non-ThryvOn cotton compared to ThryvOn cotton, as evidenced by field tests. Non-ThryvOn cotton seedlings had a greater number of immature thrips when compared to ThryvOn cotton seedlings. Non-ThryvOn cotton seedlings had a greater number of adult thrips when compared to ThryvOn cotton seedlings. Non-ThryvOn cotton seedlings had a greater number of total thrips when compared to ThryvOn cotton seedlings. Non-ThryvOn cotton seedlings had higher thrips injury when compared to ThryvOn cotton seedlings. Thrips avoidance of ThryvOn cotton appears to be a major mechanism of plant protection that has previously been observed in field trials (Graham and Stewart 2018). When thrips on ThryvOn cotton cannot avoid and are forced to feed, following a feeding event they may choose to not continue to feed resulting in starvation. The mode of action of ThryvOn on thrips is still unknown but ThryvOn technology alone was able to provide significant control of thrips.

There was no significant difference between the number of thrips on the Gaucho treated ThryvOn cotton when compared to the untreated ThryvOn cotton cultivars. This would indicate that ThryvOn does not benefit from an insecticide seed treatment. However, both ThryvOn treatments contained significantly fewer thrips than both non-ThryvOn treatments.

In summary, thrips densities and injury were reduced in ThryvOn cotton when compared to non-ThryvOn cotton. This would indicate that ThryvOn does not benefit from a supplemental thrips treatment. These observations are similar to previous and more intense studies (Graham et al. 2018). Based on these data, Arkansas should not recommend treatment of tobacco thrips in ThryvOn cotton. Because of widespread resistance in tobacco thrips to neonicotinoids and acephate ThryvOn technology has the potential to be a valuable tool in controlling this early season pest.

A greater number of TPB were found on non-ThryvOn cotton when compared to ThryvOn. TPB were recorded as small nymphs, large nymphs, adult, and totals. ThryvOn cotton had a higher density of small nymphs when compared to non-ThryvOn cotton. Non-ThryvOn cotton had a higher number of large nymphs when compared to ThryvOn cotton. A reduction in the number of large nymphs is important because they cause more damage than smaller nymphs (Cooper and Spurgeon 2013). This finding is similar to a previous and more intense study (Graham and Stewart 2018) which found a significant reduction in the number of large TPB nymphs observed. TPB exposure to Cry51Aa may not result in death of TPB but it leads to an avoidance in future feeding as well as reduces the number of large nymphs, which cause more damage than small nymphs. ThryvOn technology alone will not eliminate TPB in the field, but it does provide a considerable amount of control.

At the 1x, 2x, and 3x threshold levels, ThryvOn cotton required fewer insecticide applications than non-ThryvOn cotton plots. The need for insecticide applications was greatly reduced in all of the ThryvOn plots when compared to non-ThryvOn plots (Table 1 and 2). Due to several treatment thresholds not being initiated, results from this study indicate that the normal threshold on ThryvOn cotton provides the greatest reduction in insecticide applications for control of TPB. Season total mean TPB nymph density was reduced in the untreated ThryvOn plots when compared to untreated non-ThryvOn plots. Season total mean square retention was 14.8% higher in the untreated ThryvOn cotton compared to untreated non-ThryvOn cotton plots.

ThryvOn cotton reduced the number of TPB nymphs found in the field and improved square retention over the comparable non-ThryvOn plots. When comparing % square loss per nymph, nearly half the amount of square loss per nymph was observed on untreated ThryvOn when compared to untreated non-ThryvOn. These results indicate that the ThryvOn technology is providing fruit protection when exposed to TPB feeding.

These data indicate that ThryvOn cotton has the ability to reduce TPB applications while continuing to maintain yield when compared to non-ThryvOn treatments. ThryvOn cotton has the potential to be another valuable tool in TPB management. Previous research indicates that supplemental applications of insecticide may still be needed at times to control TPB (Graham and Stewart 2018).

When comparing yields across 2021 and 2022, ThryvOn yields were similar across treatment thresholds and control of TPB was obtained with roughly half the number of applications when compared to non-ThryvOn in two out of the three site-years. ThryvOn cotton had no yield increase when supplemental insecticide applications were made but non-ThryvOn cotton significantly increased yield at the 1x and 2x threshold. Results from this study indicate that ThryvOn may not benefit from supplemental insecticide applications. If ThryvOn cotton yields do not benefit from an insecticide application for TPB these results indicate that the current 3 TPB per 1.5 row m threshold may need to be increased for ThryvOn cotton.

Tobacco thrips has consistently been an important pest in cotton. Growers have been looking for alternative methods of control that could reduce insecticide applications. The information provided from this study shows that ThryvOn cotton has the potential, depending on technology cost, to be a valuable tool in thrips management. TPB has been the most important pest within cotton for over a decade now. Growers need alternative methods of control that provide adequate protection while also reducing the number of insecticide applications.

It is important to note that ThryvOn technology, on its own, will not provide 100% control of thrips and TPB. ThryvOn technology will allow growers to eliminate supplemental insecticide applications for proper control of thrips. Untreated ThryvOn cotton will provide considerable control against TPB but yield limiting damage from TPB feeding may still be present in the field. Growers may still be required to make some insecticide applications in moderate to high TPB pressure areas in order to protect yields but results from this study showed no yield benefit from a supplemental insecticide application for TPB. Further research is needed to determine if ThryvOn cotton will require a different treatment threshold when compared to non-ThryvOn cotton. ThryvOn technology provides growers with alternative methods of control that have the potential to minimize pest resistance issues within cotton production systems.

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Table 1. Number of insecticide applications for tarnished plant bug in ThryvOn and non-ThryvOn cotton across multiple thresholds at Marianna, Arkansas, in 2021.

Threshold Level	ThryvOn	Non-ThryvOn
1x Threshold*	2 (7/20, 8/9)	5 (7/9, 7/12, 7/20, 7/27, 8/11)
2x Threshold*	2 (7/12, 7/27)	3 (7/9, 7/12, 7/30)
3x Threshold*	1 (7/30)	1 (7/23)
3 Large Nymph+	0	1 (7/23)
6 Large Nymph+	0	0
Total	5	10

*Denotes Threshold as 6 nymphs per 3.0 row m

+Denotes Large Nymph as 4th and 5th instar nymphs

Table 2. Number of insecticide applications in ThryvOn and non-ThryvOn cotton for tarnished plant bug across multiple thresholds at Marianna and Tillar, Arkansas, in 2022.

Threshold Level	ThryvOn	Non-ThryvOn	ThryvOn	Non-ThryvOn
	Marianna		Tillar	
1x Threshold*	2 (7/15, 8/2)	5 (7/5, 7/15, 7/26, 8/2, 8/12)	2 (7/12, 7/26)	2 (7/12, 7/18)
2x Threshold*	2 (7/26, 8/12)	4 (7/15, 7/26, 8/2, 8/12)	1 7/18)	1 (7/18)
3x Threshold*	2 (7/26, /12)	2 (7/26, 8/2)	1 (7/18)	1 (7/18)
80% square retention+	0	2 (7/15, 8/12)	0	0
85% square retention+	2 (7/15, 8/12)	3 (7/5, 7/15, 8/12)	0	0
Total	8	16	4	4

*Denotes Threshold as 6 nymphs per 3.0 row m

+Denotes square retention as number of retained squares out of 25 checked

Table 3. Average number of small, large, adult, total, small to large ratio, and square retention of TPB for ThryvOn and non-ThryvOn cotton when averaged across two years. Treatments with the same lowercase letters are not significantly different according to All pairs, Tukey HSD Test (α=0.05) to separate means.

Maturity	ThryvOn	Non-ThryvOn	*F*	df	*P*
Small*	13.0 (1.1) a	17.1 (1.3) b	5.5	1,137	0.002
Large+	1.7 (0.3) a	4.4 (0.5) b	21.4	1,137	<0.001
Adult	0.4 (0.1) a	0.7 (0.2) a	2.5	1,137	0.012
Total	15.1 (1.3) a	22.2 (1.7) b	10.7	1,137	<0.001
Small to large ratio	7.6 (1.2) a	5.7 (1.3) b	10.0	1,137	<0.001
Square retention≠	86.7 (1.1) a	71.9 (0.6) b	26.5	1,137	<0.001

*Denotes small as 1st and 2nd instar nymphs

+Denotes large as 3rd, 4th, and 5th instar nymphs

≠ Denotes square retention at the first position fruiting site third node down from the top of plants until 25 sites were examined in each plot and the number of retained squares was recorded

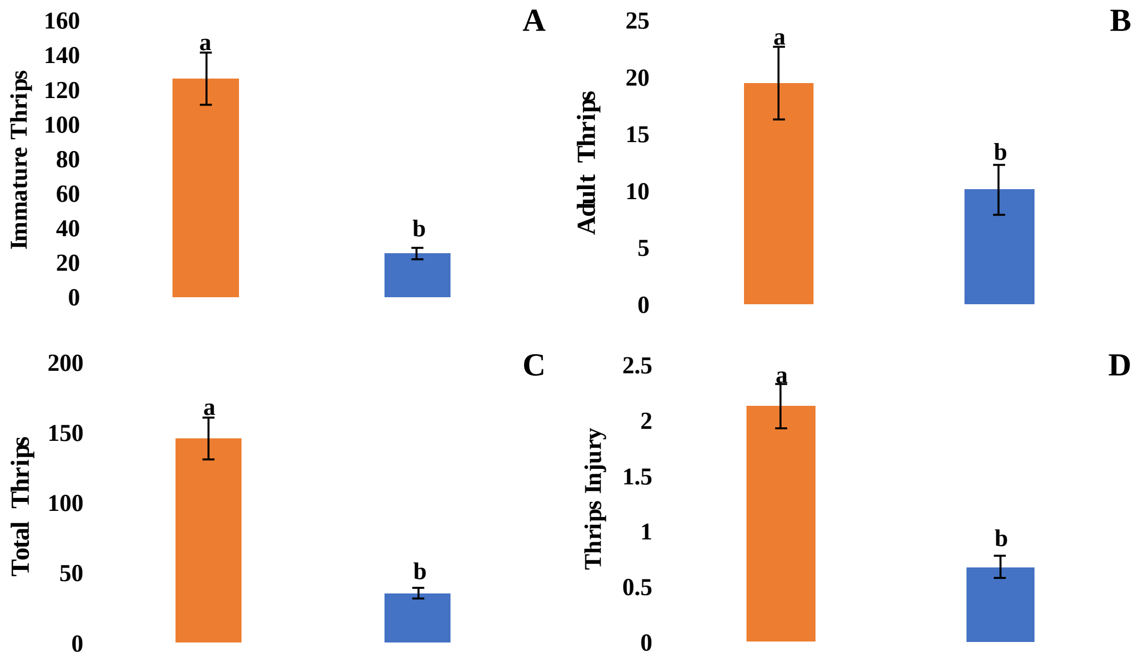

Figure 1. Number of immature thrips (A), number of adult thrips (B), number of total thrips (C), and thrips injury (D) (per 5 plants) on ThryvOn seedlings and non-ThryvOn seedlings when averaged across two years and three locations. The orange bars represent non-ThryvOn and the blue bars represent ThryvOn. Treatments with the same lowercase letter are not significantly different according to All pairs, Tukey HSD Test (α=0.05) to separate means.

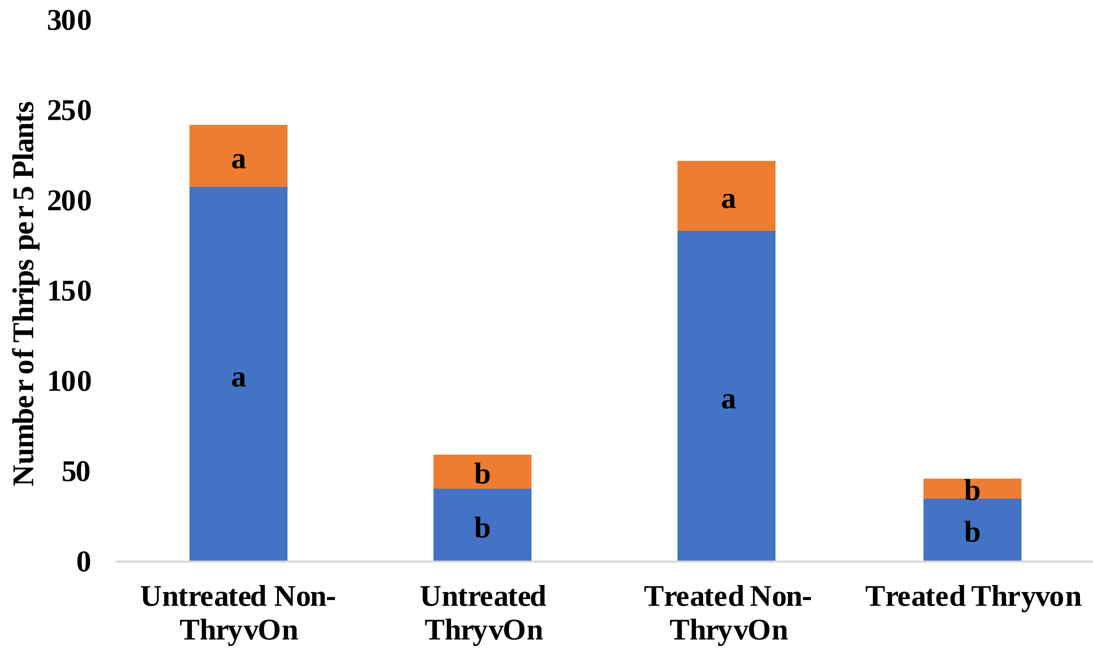

Figure 2. Effects of Gaucho seed treatment on ThryvOn seedlings and non-ThryvOn seedlings at Marianna, Arkansas, in 2021. The orange bars represent adult thrips and the blue bars represent immature thrips. Treatments with the same lowercase letter are not significantly different according to All pairs, Tukey HSD Test (α=0.05) to separate means.

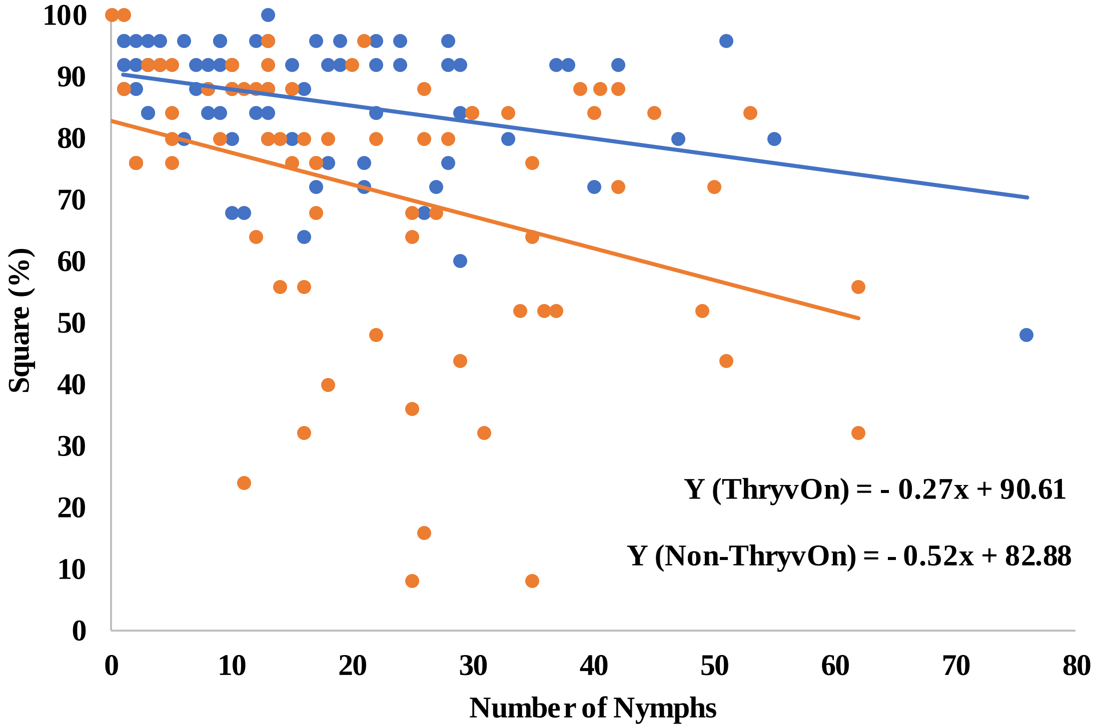

Figure 3. Average % square loss per nymph for untreated ThryvOn and untreated non-ThryvOn cotton when averaged across two years. Orange represents non-ThryvOn, and blue represents ThryvOn.

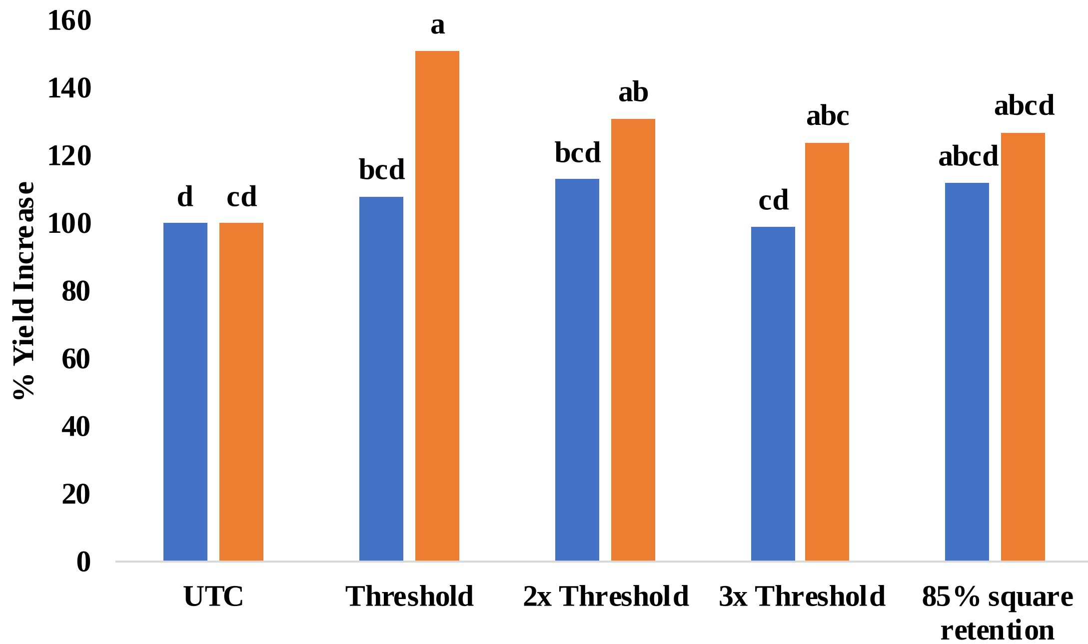

Figure 4. Average yield % increase for ThryvOn and non-ThryvOn cotton when averaged across two years. Only treatments shown were those that triggered on both ThryvOn and non-ThryvOn. Orange bars represent non-ThryvOn, and blue bars represent ThryvOn. Treatments with the same lowercase letters are not significantly different according to All pairs, Tukey HSD Test (α=0.05) to separate means.

Chapter III - Behavioral Response of Thrips (Thysanoptera: Thripidae) and Tarnished Plant Bug (Hemiptera: Miridae) to ThryvOn Technology in Cotton

Abstract

Tobacco thrips, *Frankliniella fusca* (Hinds), and Tarnished plant bug (TPB), *Lygus lineolaris* (Beauvois), are the most important insect pests of cotton, *Gossypium hirsutum*, in the Mid-South. Current control methods for thrips and TPB require insecticide applications, but with rising production costs and building resistance, growers are seeking alternative means of control. A new *Bt* toxin, Cry51Aa (ThryvOn) is being evaluated for control of thrips and TPB. Experiments were conducted in 2021 and 2022 to evaluate the behavioral response of thrips and TPB to ThryvOn technology in cotton. Controlled environment choice tests were completed in 2021 to determine the feeding preference of 50 adult tobacco thrips when presented a choice between five ThryvOn and five non-ThryvOn cotton seedlings. A significant number of adult thrips preferred non-ThryvOn cotton in controlled environment tests. More adult thrips were found on non-ThryvOn cotton when compared to ThryvOn cotton. Behavioral response tests in the field for TPB were conducted with a cage study. Adult TPB were caged on the upper 4 nodes of non-ThryvOn and ThryvOn cotton. Field evaluations of TPB showed a higher percent mortality of adult TPB on ThryvOn cotton when compared to non-ThryvOn cotton. Higher square retention was observed on ThryvOn cotton when compared to non-ThryvOn cotton. The behavioral response of these pests when exposed to ThryvOn technology will play a key role in future control strategies and resistance management. This new technology has the potential to be incorporated into future cotton insect management systems.

Introduction

Tobacco thrips, *Frankliniella fusca* (Hinds), are the most important pest of seedling cotton in Arkansas. One hundred percent of cotton acres in Arkansas are infested with Thrips (Cook 2019). Reports in 2012 concluded that thrips were the second most economically damaging insect pest in Arkansas cotton, with insecticide costs from both foliar treatments and insecticide seed treatments costing cotton growers over ten million dollars (Williams, 2012). Tobacco thrips in Arkansas are responsible for up to 84% of all thrips species found on seedling cotton (Clarkson 2014). When left untreated, thrips injury can lead to stunted growth, delayed maturity, reduced stands, and yield loss (Layton and Reed 2002, Stewart and Lentz 2010).

Control of thrips on cotton seedlings is generally achieved with use of prophylactic at-planting insecticide treatments. This practice has been widely adopted because damage can occur quickly after seedling emergence. Control is achieved through the use of in-furrow granular or liquid insecticides such as aldicarb (AgLogic 15GG®; AgLogic Chemical Company LLC; Chapel Hill, NC) or acephate (Orthene 97®; Valent USA; Walnut Creek, CA) respectively, or seed treatments such as acephate, imidacloprid (Admire Pro®; Bayer Crop Science; St Louis, MO), and thiamethoxam (Centric 40WG®; Syngenta Crop Protection, Inc.; Greensboro, NC) (Cook et al. 2011). Williams (2012) reports that over 99 percent of Arkansas cotton acres are planted with an insecticide treated seed. Residual activity of at-planting insecticides ranges from 2 to 4 weeks after planting. Traditionally there were several benefits when using seed treatments including increased vigor and equivalent efficacy to alternative methods, cheaper method of application, convenience to the grower, and reduction in equipment cost (Taylor and Harman 1990). In recent years, thrips control failures with neonicotinoid seed treatments have occurred across the Mid-South (Darnell et al. 2018) and the Southeast (Huseth et al. 2016, Huseth et al. 2017), leading to the need of new ways to manage this pest.

At-planting insecticide treatments may fail to provide adequate thrips control because of resistance to neonicotinoids, severe thrips pressure, or unfavorable growing conditions, so foliar-applied insecticide applications may be needed for optimal plant protection (Cook et al. 2020). During the year of 2012 alone, 196,000 of 235,000 (84%) hectares of cotton were treated with a supplemental foliar insecticide application for thrips control in Arkansas. On average, 4.4 foliar applications were made per hectare, resulting in a cost of > $4.3 million to Arkansas cotton growers (Clarkson, 2014). Growers need alternative methods of control while also reducing the need for additional insecticide applications.

The tarnished plant bug (TPB), *Lygus lineolaris* (Beauvois), is the key insect pest of cotton in the Mid-South. Economic damage from TPB occurs from the beginning of squaring and continues through bloom (Layton 2000). TPB is a highly polyphagous mirid that damages many economically important plants across much of the Americas and is found in all the agricultural regions of the United States. It has a large host range that includes non-crop plants, commercial flowering plants, nursery plants, fruit crops, vegetable crops, greenhouse crops, grains, and row crops. Over half of the cultivated plant species grown in the United States are listed as host plants for TPB (George, 2021). TPB feeds on cotton squares and small bolls, which reduces lint quality and yield, and also causes millions of dollars in damage annually (George et al. 2021).

Fruit retention during the pre-bloom period is critical to maximize yield. TPB infestations are managed using foliar insecticide applications; however, the TPB has developed resistance to many of the insecticides commonly used for management (Cleveland and Furr 1979, Snodgrass and Scott 1988, Snodgrass 1996, Snodgrass and Scott 2000, Snodgrass 2006, Snodgrass et al. 2009, Parys et al. 2017). Reliance on foliar insecticide applications to control Lygus populations in the transgenic cotton era resulted in the widespread development of pyrethroid and organophosphate resistance throughout the Mid-South, which in turn has affected current cotton pest management strategies (George, 2021). TPB is difficult to control with growers averaging 4-6 insecticide applications per year (Cook 2019). It is currently recommended that growers' budget approximately $247 per hectare to allow for proper control of TPB throughout the season (Watkins 2019).

Bacillus thuringiensis (*Bt*) has been documented to have insecticidal properties for over a century. Cotton varieties expressing *Bt* have been widely adopted for controlling key lepidopteran pests (Siebert et al. 2008). Baum et al. (2012) reported a *Bt* protein (Cry51Aa2) with insecticidal activity against both *Lygus hesperus* (Knight) and *Lygus lineolaris* (Beauvois). Bayer Crop Science (Monsanto company; St. Louis, MO) has developed ThryvOn cotton with Cry51Aa, making it the first cotton biotech trait that will provide season-long protection against TPB and thrip species. Graham and Stewart (2018) reported that ThryvOn cotton provided as good or better thrips control than a current, insecticide-based approach for thrips management. More recently, Krob et al. (2021), suggest that tobacco thrips have developed resistance to acephate, and other organophosphate insecticides based on bioassay data. From a management standpoint, resistant tobacco thrips will require new methods of control. Krob et al. (2021), also found that when sprayed based on TPB thresholds, ThryvOn cotton and non-ThryvOn cotton made similar yields, but the ThryvOn cotton required fewer insecticide applications.

Graham and Stewart (2018) reported that thrips avoidance of ThryvOn cotton appears to be a major mechanism of plant protection that has previously been observed in field trials. The mode of action of ThryvOn on TPB extends beyond mere avoidance (Graham 2018) but no previous research has reported the behavioral response of TPB when exposed to ThryvOn. The behavioral response of thrips and TPB to ThryvOn technology could lead to an important role in future cotton pest management strategies. The objective of this study was to evaluate the behavioral effects of thrips and TPB when exposed to ThryvOn cotton.

Materials and Methods

Thrips (No choice test)

Choice tests were conducted in 2021 at the Lonoke County Agricultural Extension Center in Lonoke, AR to determine if field populations of adult thrips have a feeding preference when presented a choice between ThryvOn or non-ThryvOn cotton. A randomized complete block design including five Thryvon (DP 2131 B3TXF; Bayer Crop Science; St Louis, MO) and five non-Thryvon (DP 2055 B3XF; Bayer Crop Science; St Louis, MO) planted pots were established. Pots were planted with either three non-ThryvOn seeds or three ThryvOn seeds. Seeds were planted into potting soil 2.0 cm below the surface and watered daily. After emergence, two seedlings were removed from each pot to leave the healthiest appearing seedling. Five non-ThryvOn seedlings and five ThryvOn seedlings were placed inside a 45.7 cm x 45.7 cm x 30.5 cm insect viewing cage and replicated in five total cages per run. This trial was replicated 8 times. A collection of wild tobacco thrips was made in a Marianna, AR cotton field. Infested cotton plants in the field were cut at the base and placed into 31.8 cm x 31.8 cm x31.8 cm bucket with cabbage and damp paper towels. Thrips were left in the 31.8 cm x 31.8 cm x31.8 cm bucket with a 100-micron lid for 24 hours to ensure healthy specimens were used during the experiment. An aspirator was used to collect fifty adult tobacco thrips into a test tube and a 100-micron screen was taped over the opening. One test tube with 50 tobacco thrip adults were placed within each cage. The mesh lid was removed from the test tube before sealing the cage and thrips were left in the cage for 24 h. After 24 h, the seedlings were collected from each cage by cutting the seedling at the soil surface and placing in jars with a 70% ethyl alcohol and water mixture to collect adult tobacco thrips. Each plant was removed from the jar and rinsed with 70% ethyl alcohol. Following seedling removal, the jar was emptied into a glass container topped with filter paper to collect adult thrips. Jars were then rinsed with 70% ethyl alcohol over the filter paper to collect any remaining thrips. The filter paper was then rinsed with 70% ethyl alcohol into a gridded 100 mm x 15 mm petri dish and adult thrips were counted underneath a microscope using 10-20x magnification.

Controlled environment no choice tests were used to test whether more adult thrips were found on either ThryvOn seedlings or non-ThryvOn seedlings. Adult thrips preference data were analyzed using JMP pro version 16.2.0. A one-way analysis of means was conducted with an all-pairs Tukey HSD analysis (α=0.05

Tarnished Plant Bugs (Cage Study)

A cage study to determine relative efficacy and plant injury between non-ThryvOn cotton and ThryvOn cotton was conducted in 2022 at the Lon Mann Cotton Branch Research Station located in Marianna, AR. On 11 May, eight rows of non-ThryvOn cotton (DP 2055 B3XF; Bayer Crop Science, St Louis, MO) were planted next to eight rows of ThryvOn cotton (DP 2131 B3TXF; Bayer Crop Science, St Louis, MO). Plot sizes were 8 rows (0.97 m centers) x 300 m and left untreated throughout the season. TPB were collected with 38.1 cm diameter sweep nets on wild hosts (tall goldenrod (*Solidago altissma* L.), pigweed (*Amaranthus* spp.), and white heath aster (*Aster pilosus*)) and placed in a 30.5 x 30.5 x 30.5 bug dormer (BioQuip Products; Rancho Dominguez, CA; MFG #1466AV) with heads of wild hosts. This study was conducted after the third week of squaring until 5 NAWF. At the time of trial initiation, cotton plants were thoroughly examined to insure no TPB were on the plant and no visual injury was present. After inspection a 120-micron mesh paint strainer (12.7 cm x 12.7 cm x 40.6 cm) (Lowe's Companies, Inc.; Mooresville, NC; MFG #11522) was placed around the top 4 nodes of the plants. Cages were then infested with 0, 1, or 3 adult TPB using an aspirator. Infested cages were carefully placed over the top 4 nodes of the cotton plant and secured around the stem with a wire tag. Infestation level was then labeled on the tag. The experiment was designed as a randomized complete block design with ten replications for each infestation level in ThryvOn and non-ThryvOn cotton and replicated five times throughout the growing season. After seven days, cages were removed by cutting the stem one inch below the cage. Through visual observations, the number of dead TPB that had fallen to the bottom of the cage vs alive TPB within the cage was recorded. Next, the cage was opened, and the plant material was removed. The number of total fruiting positions was recorded and the presence or absence of squares at each position was recorded.

Cage studies on blooming ThryvOn and non-ThryvOn cotton were done to test the Mortality of adult TPB and square retention when adult TPB were caged on the top four nodes of blooming cotton. TPB behavioral response data were analyzed using JMP pro version 16.2.0. A one-way analysis of means was conducted with an all-pairs Tukey HSD analysis (α=0.05).

Results

Thrips

Non-ThryvOn cotton seedlings had a greater number of adult thrips when compared to ThryvOn cotton (Fig. 1). A difference between ThryvOn and non-ThryvOn was observed for thrips feeding preference (F=72.8; df=1,79; P<0.001). When sampling thrips on cotton seedlings in a controlled environment, on average, non-ThryvOn cotton seedlings (7.5) had 4.4-fold higher adult thrips numbers than ThryvOn cotton seedlings (1.7) (Fig. 1).

Tarnished Plant Bug

TPB mortality was greater when caged on ThryvOn cotton than when caged on non-ThryvOn cotton (F=28.9; df=5,299; P<0.001). ThryvOn cotton (56%) had greater % mortality than the non-ThryvOn cotton (42%) when 1 TPB were caged on plants (Fig. 2). ThryvOn cotton (56.7%) had greater mortality than the non-ThryvOn cotton (48%) when 3 TPB were caged on plants (Fig. 2). There were no differences in percent mortality when ThryvOn cotton was caged with 1 or 3 TPB. ThryvOn cotton (9.6%) had fewer squares missing than the non-ThryvOn cotton (14.5%) when 1 TPB were caged on plants (F=4.6; df=5,299; P=0.006) (Fig. 3). ThryvOn cotton (8.2%) had fewer squares missing than the non-ThryvOn cotton (15.8%) when 3 TPB were caged on plants. There were no differences in square loss when cotton was caged with 1 or 3 TPB.

Discussion

When presented with a choice between ThryvOn and non-ThryvOn cotton seedlings, adult tobacco thrips preferred feeding on non-ThryvOn cotton. ThryvOn seedlings had a lower number of adult thrips after sampling (60%) when compared to non-ThryvOn seedlings. Graham and Stewart 2018 reported a similar trend when completing field and greenhouse choice studies. With ThryvOn's activity on thrips being related to avoidance, it may be a valuable tool in insecticide resistance management. Adult thrips may try to avoid ThryvOn cotton and move into non-Thryvon fields or to a suitable wild host, but when they have no other choice, they may still feed on the plant but avoid future feeding and eventually starve to death. ThryvOn technology alone will not eliminate thrips pressure, but it will relieve pressure in problematic fields and will eliminate the need for supplemental foliar insecticide applications.

Cage studies of TPB resulted in higher mortality as well as increased square retention for ThryvOn cotton when compared to non-ThryvOn cotton. Cry51Aa is a toxin, but it may not provide enough control to completely eliminate foliar applications for moderate to high populations of TPB. Also, higher square retention was observed on ThryvOn cotton regardless of the infestation level. Square loss is still observed in ThryvOn cotton; however, it is to a lesser degree than non-ThryvOn cotton under similar TPB densities. In order for the Cry51Aa toxin to take effect, TPB is required to feed on the plant in order to consume the toxin. The act of TPB feeding on the cotton plant will result in some injury but it is significantly less than the injury observed on non-ThryvOn cotton.

Tobacco thrips have consistently been an important pest in cotton. Growers have been looking for alternative methods of control that could reduce insecticide applications as well as increase yield. The information provided from this study shows that ThryvOn cotton has the potential, depending on technology cost, to be a valuable tool in thrips management. TPB has been the most important pest within cotton for over a decade now. Growers need alternative methods of control that reduce the number of insecticide applications. These data suggest that ThryvOn has the potential to be a valuable tool in controlling TPB.

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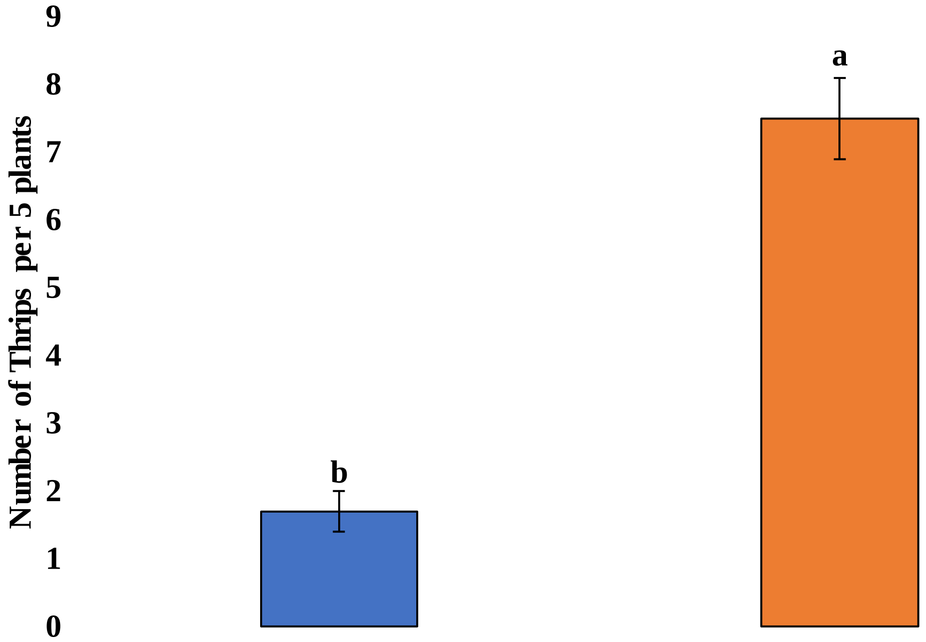

Fig 1. Results from a 2021 controlled environment choice test in Lonoke, AR to determine if field populations of adult thrips showed a preference for non-ThryvOn cotton compared to ThryvOn cotton. The orange bar represents non-ThryvOn and the blue bar represents ThryvOn. Treatments with the same lowercase letter are not significantly different according to All pairs, Tukey HSD Test (α=0.05) to separate means.

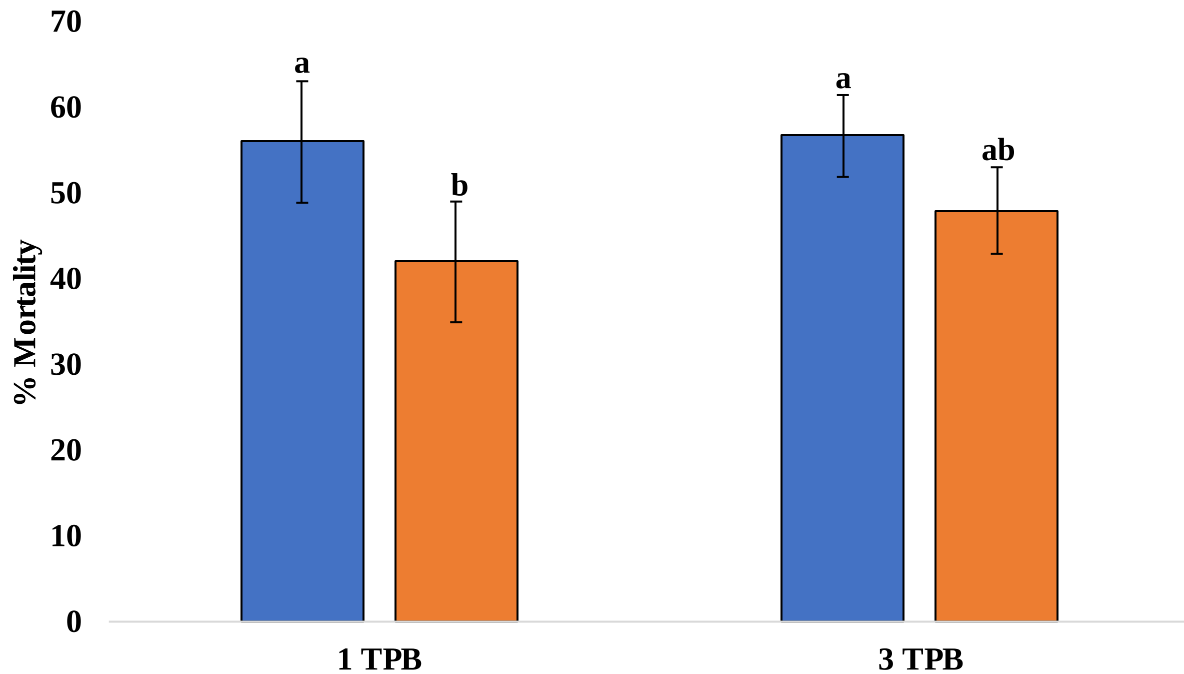

Fig 2. Results from a 2022 cage study in Marianna, AR comparing percent mortality (# Dead/Total TPB) in ThryvOn and non-ThryvOn cotton 2022. Orange bars represent non-ThryvOn and blue bars represent ThryvOn. Treatments with the same lowercase letter are not significantly different according to All pairs, Tukey HSD Test (α=0.05) to separate means.

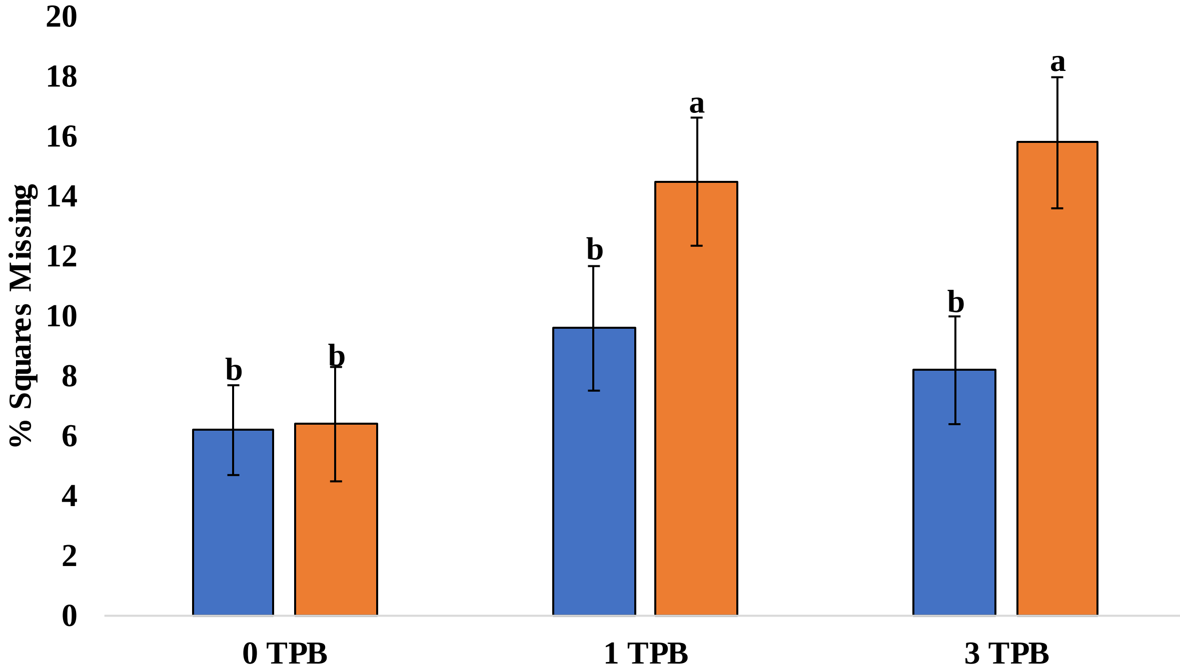

Fig 3. Results from a 2022 cage study in Marianna, AR comparing % squares missing (# damaged/Total squares) in ThryvOn and non-Thryvon cotton 2022. Orange bars represent non-ThryvOn and blue bars represent ThryvOn. Treatments with the same lowercase letter are not significantly different according to All pairs, Tukey HSD Test (α=0.05) to separate means.

Chapter IV – Conclusion

The goal of this research was to evaluate ThryvOn technology for control of tobacco thrips and TPB in cotton. The first objective evaluated the impact of ThryvOn on tobacco thrips compared to non-ThryvOn cotton with and without a seed treatment. Results indicated that within an insecticide free system, ThryvOn seedlings reduce thrips densities compared to non-ThryvOn regardless of the presence of an insecticide seed treatment. ThryvOn seedlings also had reduced feeding injury when compared to non-ThryvOn seedlings. No differences in thrips densities were observed between untreated ThryvOn and insecticide seed treated ThryvOn. Based on this data, tobacco thrips should not be treated on ThryvOn cotton in Arkansas.

The second objective evaluated various thresholds for treating ThryOn cotton. Based on current threshold recommendations of 3 TPB/1.5 row m, 1 to 5 insecticide applications were needed to manage TPB in both ThryvOn and non-ThryvOn. ThryvOn cotton treated at normal threshold never required more than 2 applications while non-ThryvOn cotton averaged 5 applications. A significant reduction of TPB on Thryvon cotton was also observed throughout the season which lead to a reduced need for insecticide applications. Untreated ThryvOn cotton provided higher square retention when compared to untreated non-ThryvOn cotton. As the threshold increased, yield decreased in non-ThryvOn plots while ThryvOn plots did not see a reduction in yield. Yields in unsprayed ThryvOn were no different than any of the sprayed ThryvOn treatments. At current threshold recommendations, ThryvOn cotton yielded similar or better than non-ThryvOn cotton with fewer insecticide applications. Further research is still warranted to determine the TPB threshold for ThryvOn cotton. ThryvOn did not benefit from supplemental insecticide applications in this study meaning the threshold has potential to be raised.

The third and fourth objectives of my research focused on the behavioral response of tobacco thrips and TPB when exposed to Thryvon technology. Adult thrips in the greenhouse consistently avoided ThryvOn seedlings when presented with a choice between ThryvOn and non-ThryvOn seedlings. Adult thrips may try to avoid ThryvOn cotton and move into non-Thryvon or to a suitable wild host, but when they have no other choice, they may still feed on the plant but avoid future feeding and eventually starve to death. In the TPB field cage study there was greater mortality on ThryvOn cotton when compared to non-ThryvOn cotton. Greater square retention was also observed in ThryvOn cotton when compared to non-ThryvOn cotton.

From a thrips standpoint, immature and adult thrips will still be observed on ThryvOn cotton but in reduced numbers compared to non-ThryvOn cotton. No benefits were observed when ThryvOn was treated with an IST. Growers should not add any additional insecticide applications to ThryvOn for thrips control. ThryvOn also reduced the number of insecticide applications needed to control TPB in the field but more research is needed to determine a threshold. In conclusion, ThryvOn technology is a valuable tool for control of thrips and TPB and will provide growers with alternative methods of control. This should benefit future cotton insect pest management systems.