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Evaluation of Bt Cotton Cultivar Efficacy and Economic Impact on Cotton Yield with and without a Foliar Insecticide Application

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Evaluation of *Bt* Cotton Cultivar Efficacy and Economic Impact on Cotton Yield with and without a Foliar Insecticide Application

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Abstract

Helicoverpa zea is the second most damaging insect pest of cotton in Arkansas. It is becoming more difficult to control in the United States due to recently documented resistant to dual gene cultivars. The objective of this thesis was to develop a better understanding of the efficacy of several *Bt* cotton technologies and foliar insecticides used to manage *H. zea*. Experiments examined damage in current commercial non-*Bt*, dual gene, and three gene cotton cultivars. Other experiments evaluated if *H. zea* could detect concentrations of chlorantraniliprole and residual of chlorantraniliprole in cotton for an extended period after application. These studies suggest that chlorantraniliprole has the potential to provide control of *H. zea* up to 28 days after treatment, based on lab analysis of residual concentrations in multiple fruiting structures. Results from both studies suggest that *H. zea* was not able to detect chlorantraniliprole at the tested concentrations. Dual gene appears to provide adequate control of *H. zea* under low pressure. However, dual gene alone may not provide satisfactory control of *H. zea* when pressure is moderate or greater and supplemental foliar insecticide applications may be required. Three gene cultivars appeared to provide sufficient control of *H. zea* but should still be monitored to prevent yield loss. Grower's planting dual gene cultivars should budget at least one application of a diamide insecticide to prevent yield loss. Information was collected from the Arkansas Field Crops Enterprise Budget to compare dual gene and three gene production and input cost. Compared to dual gene cotton, three gene cotton reduces insecticide use, lessens the amount of diesel used, decreases time spent in the field, and has a higher seed cost per acre. A simulated scenario was conducted to determine the profit and breakeven percentage for cotton growers. The simulated profit and breakeven percentage was calculated using the average yield, average market price of cotton, and the average expenses per acre. This simulation showed what

growers can expect to profit or if they will breakeven based on the cotton cultivar or if a foliar insecticide application is used. Results from these experiments will be important for refining management recommendations for *H. zea* in *Bt* cotton.

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CHAPTER I

INTRODUCTION

Cotton

The genus *Gossypium* includes 49 species, including some recently recognized from Africa/Arabia (Vollesen, 1987) and Australia (Fryxell, 1992). Cotton is a woody perennial with an indeterminate growth habit and believed to have the most complex growing structure of any major field crop (Mauney, 1984). Although numerous species of cotton exist, only limited species are significant in the agricultural industry. Upland cotton, *Gossypium hirsutum* (L.), is a perennial shrub grown as an annual plant that is predominantly grown throughout 17 southern states accounting for 97% of the United States cotton production with the additional 3% grown in Pima cotton, *Gossypium barbadense* (L.) . The United States produces the third greatest amount of cotton in the world, behind India and China (Meyer & Dew, 2022). In 2022, the United States produced 14.2 million bales of cotton (Meyer & Dew, 2022). Arkansas currently ranks fourth in cotton and cottonseed production in the United States, only behind Texas, Georgia, and Mississippi. Cotton was ranked as the seventh most valuable agricultural commodity within Arkansas in 2022 (Studebaker, 2021). Recent upland cotton planted in the United States for 2022 was estimated at 5,058,570 ha (USDA, 2022), with only 3,188,923 being harvested . The State of Arkansas accounted for 194,249 ha (2021) of the total plant (USDA, 2022). The value of production of upland cotton lint in the state of Arkansas during 2021 was $442.8 million (Nseir & Honig, 2022).

Growth and Emergence

In Arkansas, cotton is planted mid to late April and into the May dependent on soil temperatures. Soil temperature should be checked mid-morning at the depth of planting for three

consecutive days. A soil temperature of 68 °F at planting depth during the three days is best (Robertson et al., 2022). Following planting date should be a favorable five-day forecast to enhance stand. A general recommendation for seeding rate is 69,160 seeds per hectare. However, seeding rate can be dependent on soil type, with Sandy and Silt loams needing 81,510 seed per hectare (7.9 seed/m) and Clay loams needing 101,270 seed per hectare (9.9 seed/m) (Robertson et al., 2022). If cotton is planted in late May or early June, seeding rates should be increased by 10% (Robertson et al., 2022).

Germination begins as the seed absorbs water and oxygen after planting. Water swells the dormant tissues and cell growth and division begin to take place (Ritchie, 2007). Under favorable conditions for germination, the radicle emerges through the micropyle within 2-3 days (Oosterhuis, 1999). Root growth is rapid despite the early season growth of cotton above ground appearing slow. The radicle, which becomes the taproot, can reach depths up to 25.4 cm before the cotyledons emerge (Ritchie, 2007). Environmental conditions can obstruct early season root growth, such as low soil temperature, low soil pH, hard pans, herbicide injury, and soil moisture can prevent root growth and development. Root development during the early vegetative stage may proceed at the rate of 1-5 cm/day, depending on soil conditions, such that the roots may be 1.0 m deep when the above ground portion is only 0.35 m (Oosterhuis, 1999).

Germination and emergence require between 50 and 60 heat units (Oosterhuis, 1990). A dependable method used to predict cotton growth and development is heat unit estimation. The base temperature needed for cotton growth is 60°F . Daily heat units are calculated by adding the daytime high and low temperatures, dividing by 2, and then subtracting the base temperature (Oosterhuis, 1990). Understanding heat units can help to predict what stage the cotton is in, as well as how much time is needed until the next stage (Main 2012). Cotton requires 2,600 heat

units from planting to harvest to reach full maturity, which is normally 130-160 days (Oosterhuis, 1990). Seedling emergence normally takes place 4-14 days after planting (Ritchie, 2007). At the soil surface, the cotyledons are pulled through the soil surface, they unfold to expose the growing point. Before they unfold, they supply stored food to the germinating seedling (Ritchie, 2007). After emergence and exposure to light, the cotyledons become green due to chlorophyll and are capable of photosynthesis (Oosterhuis, 1999). After seedling establishment, the first true leaf appears above the cotyledons. The first true leaf shifts the plants primary energy source from storage to photosynthesis and signals the transition from emergence to vegetative growth.

Two different types of branches form on the plant, monopodial (vegetative) and sympodial (fruiting branch). These two types of branches are dependent on the axillary bud associated with each true leaf. Vegetative branches are morphologically like the main stem since they have only one meristem. Because vegetative branches have only one meristem, they grow straight and erect, much like the main stem (Ritchie, 2007). Vegetative branches themselves do not bear flowers and fruits, but axillary buds borne along the vegetative branches may form fruit bearing branches (Oosterhuis, 1999). The structure of the fruiting branches differs from vegetative branches with the addition of multiple meristems and a “zig-zag” growth habit. This “zig-zag growth habit is created by the stop-and-go growth of the fruiting branch. Once a fruiting bud forms, the initial growth of a fruiting branch is terminated. However, the growth of an axillary meristem is initiated by the fruiting branch (Ritchie, 2007). Typically between 45 and 60 heat units are needed for production of each additional main stem node (Oosterhuis, 1990). The rate of production of new vegetative leaves, new fruiting branch sites, is extremely dependent on temperature, and is very sensitive to water stress. At some point the leaf area begins to exceed

the capability of the root system to explore new soil volume or to absorb and transport water to the stomates (Mauney, 1986). In a study, Mauney et al., (1978), noted a reduction of vegetative production two weeks before flowering in cotton plants under no stress. At this point it can be suggested that the plant switches primary energy sources to reproduction. Common environmental factors such as over-fertilization, temperature, water stress, and insect or disease pressure can affect the growth of vegetative leaves which then hinders the development of reproductive branches (Mauney, 1986; Ritchie, 2007).

As the cotton plant is undergoing the reproductive cycle, it goes through several distinct stages before it can reach maturity. Floral buds appear first as small, green, pyramidal structures known as squares (Oosterhuis, 1999). A square consists of three large green bracts which forms a pyramid like shape that surround the flower bud. The first square is typically visible on node 5 to 7 about 35 days after planting, with a flower bloom occurring approximately 21 days after the first square appears (Ritchie, 2007).

The next stage of the reproductive cycle is the flowering stage. This is a vital step in cotton production because pollinated flowers form cotton bolls. The flowering process progresses over several days. Details such as bloom age can be estimated by bloom characteristics (Ritchie, 2007). Flowers are a creamy white on the day of opening. Pollination of the flower tends to occur within a few hours after the white flower opens (Oosterhuis, 1999). As the flowers near maturity, it will change to a pink-like color on the second day, and a reddish color on the third day. The flowers dry and abscise at the base 5 to 7 days after it appears. Once the flower falls from the plant, the developing boll is exposed. Boll development takes place through three distinct phases, enlargement, filling, and maturation, which takes about roughly 50 days to complete (Ritchie, 2007). During the enlargement phase, fibers are produced on the seed are

stretching and the maximum volume of the boll and seeds are acquired in a short three weeks' time (Ritchie, 2007). As the fourth week of flowering approaches, fiber elongation concludes, and secondary wall formation of the fibers begins. This progression is known as fiber filling, or deposition. As the boll reaches its full size and weight, the maturation process begins. During this phase, fiber and seed maturation take place and boll dehiscence occurs (Main, 2012). When the boll dries, the capsule walls shrink unevenly causing a split which leads to the opening of the boll. To grow cotton from seed to harvest across the midsouth a minimum of 150 days under optimum conditions are needed.

Insecticide applications are terminated based upon crop growth stage. Knowing when a cotton crop is near cutout can help growers make effective end-of-season decisions. Cutout is a turning point when terminal growth slows. The number of nodes above white flower (NAWF) should be monitored during the bloom period. The number of nodes above the upper most first position white flower on a cotton plant should be counted to determine NAWF. When the average NAWF value of 5 (NAWF=5) is reached, the field is considered to be cutout (Robertson et al., 2022). Research shows the last population of bolls that will effectively contribute to yield will be represented by white blooms present at cutout (Owen, 2015). Cutout is reached in cotton when the number of nodes above the uppermost white bloom on the first position is equal to five (NAWF=5) in late July or early August in Arkansas (Robertson et al., 2022). Once cutout is reached, the number of heat units that are accumulated are based on heat unit estimation (DD60s). *H. zea, Helicoverpa zea (Boddie),* and tobacco budworm, *Heliothis virescens* (F.), require cutout plus 350 DD60s, while Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), requires cutout plus 250 DD60s (Robertson et al., 2022). When bolls obtain 350 to 400 heat units or DD60s, they are less likely to suffer economic damage from tarnished plant

bugs (nymphs or adults) or from budworm/*H. zea* larvae that emerge after this point. Alternatively, heat units are accumulated based on historical weather for specific production regions, known as seasonal cutout. Much of the later planted cotton that is still maintaining 5+ NAWF, seasonal cutout will be used to determine the last white blooms present that will contribute to yield. Seasonal cutout is a day in which the probability of a new flower developing into a boll declines to 50%, meaning it is unlikely the fruiting positions which develop after this date will contribute to yield (Raper, 2022). Seasonal cutout dates are based upon which growing region or area of the state growers might be in. Seasonal cutout dates in Arkansas range from August 8th to August 21st depending upon parts of the state. Northern cotton growing portions of the state will be closer to the August 8th seasonal cutout date, while southern portions of Arkansas will be near August 21st.

Insect Complex

From emergence until harvest, various pests attack the roots, leaves, stems, or fruit (squares, blooms, and bolls) of cotton (Boyd et al., 2004). Economic threshold levels have been established for many cotton pests. A threshold infestation is the point at which control measures are needed to prevent the target pest from reaching its economic injury level (when control costs equal damage caused by the pest (Boyd et al., 2004). From emergence to fourth true leaf, thrips belonging to the genus *Franklinella* are important pests. In the mid-South production area, the tobacco thrips, *Frankliniella fusca*, is the predominate species that occurs on cotton (Wann, 2015). Injury from thrips is exhibited by silvering of lower leaf surfaces as well as the cupping of leaves (Cook et al., 2011). When such injury occurs within the terminal bud, on developing leaves and fruiting structures, the damage usually results in delayed maturity and yield losses (Cook et al., 2011). Mid to late season pests of cotton include several fruit-feeding insects,

including *H. zea*, tobacco budworm, cotton aphid, *Aphis gossypii* (Glover); two spotted spider mite, *Tetranychus urticae* (Koch); and several defoliating caterpillars (Allen et al., 2018). The tarnished plant bug is considered the most economically damaging pest of cotton in the mid-southern United States (George et al., 2021). The introduction of transgenic crops for the control of larvae in the *Heliothine* complex and eradication of the boll weevil, *Anthonomus grandis* (Boheman), from much of the United States led to greatly reduced pesticide use in cotton fields, which allowed *L. lineolaris* to emerge as a new primary pest of cotton in the mid-southern United States (George et al., 2021).

H. zea **Biology**

Helicoverpa zea is an economically important polyphagous pest found in the Western Hemisphere (Randall G. Luttrell & Ryan E. Jackson, 2012). *H. zea* feeds on several important crops such as cotton, soybean, *Glycine max* (L.)., sorghum, *Sorghum bicolor* (L.), and many vegetable crops causing significant economic loss. *H. zea* belongs to the Order Lepidoptera and in the family Noctuidae. While the insect is not thought to overwinter at latitudes greater than 40° (Hardwick, 1965), *H. zea* is also a common pest in northern regions of the United States as a result of yearly migration. As a polyphagous pest, *H. zea* has three common names approved by the Entomological Society of America (corn earworm, tomato fruit worm, and bollworm), in addition to being known as sorghum head worm, soybean pod worm, and vetch worm (Reay-Jones, 2019). Formerly thought to be part of one species with the old-world bollworm, *Helicoverpa armigera* (Hübner), the taxonomy was clarified by Hardwick (1965) with a description of the two distinct species. The insect has since received considerable attention due to its damage to numerous economic crops, but particularly as a pest susceptible to transgenic corn hybrids and cotton cultivars expressing *Bacillus thuringiensis* (*Bt*).

Lepidopterans experience complete metamorphosis (Rolff et al., 2019). Complete metamorphosis consists of four phases egg, larva, pupa, and adult. The lifecycle of *H. zea* takes roughly 30 days. The adult stage is a stout bodied, brownish to buttery-yellow moth with a wingspan of about 3.175 to 3.81 cm (Porter, 2020). Small spots are sometimes visible on the forewings, while dark outer-marginal bands and brown disc-shaped spots are found on the dorsal surfaces of the underwings (Hardwick, 1965). Eggs are deposited individually on a host plant. A single female can lay 1000 eggs in a period of 10 to 20 days (Bishop, 1907). *H. zea* eggs have an approximate dimension of 0.5 mm length by 0.5 mm width and vary in color from white after they are laid to yellow near larval hatch (Hardwick, 1965). Eggs are laid on the leaves of host plants and stalks or silks of corn. Maturation of the egg can be noticed, eggs hatch within 3 to 4 days with the newly laid egg being pale green. The eggs later progress to a yellowish or white color before turning gray. The hatched larvae migrate to a feeding site where they feed on leaves, ears of corn, soybean pods, or fruiting structures. There are five to six larval instars (Porter, 2020). The first instar is about 1.5 mm long and the 6th instar can grow to 44.5 mm long (Porter, 2020). *H. zea* larvae vary greatly in color ranging in from light green or pink to dark brown or nearly black. Alternating light and dark stripes run the length of the body (Cook, 2004). A yellowish band is often found on the side of the larvae, and the band contains the dark, circular spiracles, respiratory openings found on the insect's body. The larval stage lasts 12 to 15 days during the warm part of the growing season and is longer when it is cooler. *H. zea* larva become more aggressive and cannibalistic as they mature. The volume of injury done depends on larval instar because as the larva becomes older, the amount of fruit consumed increases (Wilson, 1982). According to Wilson (1982), a fifth instar larva can damage up to 11 times more fruit than a first instar. Fully grown larvae drop to the ground where they burrow to create a pupal cell. The

pupa is also like many other Noctuid species, with a shiny red to dark brown body. The pupal stage lasts 13.5 days for males and 12.6 days for females (Hardwick, 1965). The insect overwinters in the soil as diapausing pupae (Hardwick, 1965). The moths emerge in early-April and late-May in Arkansas (Slosser, 1975). Early oviposition sites include numerous wild hosts. Early oviposition depends on the host species abundancy and distribution. Typical host plants include crimson clover *Trifolium incarnatum* (L.); hairy vetch *Vicia villosa* (Roth); and corn *Zea mays* (L.); which becomes a primary food source after host plants fully mature.

H. zea **as a Pest in Cotton**

H. zea larvae prefer specific feeding sites on cotton plants (J. Gore, 2000). Feeding typically takes place on the reproductive structures of cultivated and wild host plants. Cotton losses from *H. zea* damage is more likely to be in yield, rather than in lint quality (Adkisson, 1964). Heliothis egg distribution on cotton can differ significantly. Possible factors influencing this variation include species, cotton varieties, and environmental conditions and their effects on the insects and host plants (Farrar Jr & Bradley Jr, 1985). *H. zea* oviposition was originally assumed to primarily occur in the upper region of the plant (Gore et al., 2002). Braswell et al. (2019), found the distribution of eggs within the cotton canopy varied weekly throughout the season. During stages of flowering, moths oviposit lower in the plant canopy on foliage, flower petals, and the bracts of reproductive structures (J. Gore, 2000). Larvae hatching from eggs that were oviposited lower in the plant canopy feed on flowers and bolls, often leading to their abscission (Farrar Jr & Bradley Jr, 1985) . Hatched larvae feed on surrounding plant tissue which includes the terminal, squares, flowers, and bolls. *H. zea* larvae have been observed moving away from young tissues in the terminal of cotton plants and ultimately settling in flowers where *Bt* protein expression is lower (Godbold et al., 2022). Pascua (2002), found that older larvae

move extensively within the plant preferring to feed on large squares and bolls at the middle and lower part of the plant where protein expression is lower. Research shows that yield loss from damage depended on location of the injury, as well as growth stage of the plant. Bolls that have developed for 15-18 Days after anthesis generally will not abscise from the plant (Guinn, 1982, 1986). However, yield losses may still occur from *H. zea* feeding on bolls that do not abscise (J. Gore, 2000).

H. zea infested 2.7 million hectares or 65% of total U.S. planted cotton in 2021 with 360,000 of those hectares being treated with insecticide. In Arkansas, 100% of cotton hectares were infested and 11.7% of the hectare needing an insecticide application (Cook, 2022). There were 38,000 bales of cotton lost to *H. zea* in 2022, making *H. zea* the second most economically damaging pest of Arkansas cotton behind the tarnished plant bug (Cook, 2022). In Arkansas, an average of 1.2 foliar insecticide applications were need to control *H. zea* , costing $4.99-$20 per hectare (Cook, 2022).

Primary Control Methods in Cotton

Chemical Control

Prior to the production of genetically modified cotton, chemical control was the primary method used to manage *H. zea.* Many insecticides have been used over the years to decrease *H. zea* densities in cotton. Most of the insecticides currently used against agricultural pests are neurotoxic compounds such as pyrethroids, organophosphates, and carbamates that result in relatively inexpensive and reliable control (Haynes, 1988). Pyrethroids constantly proved to reduce the *H. zea* larvae population, becoming popular due to their cost effectiveness and broad-spectrum control across various crop systems (Brown et al., 1998; Pietrantonio et al., 2014).

However, the efficacy of pyrethroids began to decline due to developing resistance, caused by overuse (Stadelbacher et al., 1990; Hopkins, 2010; Pietrantonio et al., 2014). Resistance originally was noticed in pyrethroids, but now carbamates and organophosphates defined by the Insecticide Resistance Action committee (IRAC) (IRAC, 2022). Insect growth regulators (IGRs) and spinosyns which are physiologically and ecologically selective, have been investigated alternatives or complements to organophosphates and carbamate insecticides (Rafiei et al., 2008). In order to delay or avoid the rapid development of resistance in treated pest, new insecticides were created with novel modes of action. chlorantraniliprole was introduced in 2008, and is in the IRAC Mode of Action Group 28 family (IRAC, 2022). Unlike previous listed insecticides, chlorantraniliprole is relatively harmless to beneficial arthropods and is found to have a low mammalian toxicity (Lahm et al., 2005). The diamides have replaced many of the previous chemistries used to control lepidopteran pests in cotton and other row crops in the Southern United States. The use of broad-spectrum insecticides had direct impacts on the total spectrum of arthropods associated with cotton agroecosystems. In 1995, one year before commercial deployment of Bollgard cotton, insecticide use and outbreaks of uncontrollable pest populations were common (Randall G Luttrell & Ryan E Jackson, 2012). Mild winters across the cotton belt during 1994 and 1995 allowed insect pest to survive the winter far north of their normal range and in larger populations (Carter, 1995). Heavy insect pest populations occurred early in the growing season, causing growers to have no choice but to protect their cotton crop with numerous insecticide applications. Applications of products were either a single product or mixtures to help manage pest numbers. The natural enemy complex declined as a result of early season insecticide treatments made for thrips, aphids, June populations of tobacco budworms, plant bugs, and boll weevil eradication treatments (Carter, 1995). To combat resistance, an

insecticide rotation strategy is currently recommended to avoid the development additional resistance. Commercial *Bt* cotton varieties are also being used to aid in controlling lepidopteran pest in cotton with synthetic insecticides. Insecticides are now applied supplementally to control high populations of *H. zea* in transgenic cotton (Randall G Luttrell & Ryan E Jackson, 2012).

Transgenic Cotton Cultivars

Transgenic cotton cultivars were commercially introduced in 1996 with the launch of Bollgard® (Monsanto Co., St. Louis, MO), was the first transgenic cotton cultivar commercially introduced to control lepidopteran insect pest. This technology was quickly adopted by the US cotton industry, especially in the southeast and midsouth regions, where the new cultivars provided control of the insecticide resistant tobacco budworm (Randall G Luttrell & Ryan E Jackson, 2012). *Bt* cottons dramatically changed the approach to insect management on cotton, especially throughout the Mid-Southern US where populations of insecticide-resistant tobacco budworm previously limited yield and multiple applications of insecticide were required (Luttrell, 1994; Naranjo & Luttrell, 2009). Transgenic *Bt* cotton provided control of tobacco budworm that caused significant economic damage to US cotton (Naranjo & Luttrell, 2009). Commercial use of *Bt* cotton caused a shift in the use of broad-spectrum insecticides, allowing a decrease in the number of applications. In 1995, growers averaged 10 insecticide applications per year, compared to 2001 they averaged 4 insecticide applications per year (Williams, 1995, 2001). *Bt* cotton offers value to growers in situations where insecticide applications cannot be made, such as in field that do not allow the proper operation of air or ground sprayers, in remote fields, or weather events (Hardee et al., 2001). Reduced input costs and less insect pest damage result in higher yields, which offset the technology fee and favor *Bt* cotton.

Commercially, growers had access to Bollgard, which expressed the Cry1Ac protein isolated from *Bacillus thuringiensis* (*Bt*), which is a ubiquitous Gram-positive, rod-shaped and sporulating bacterium that has been isolated worldwide from a great diversity of ecosystems (Palma et al., 2014). This protein was well characterized, considered safe, and specific to the lepidopterous class of insects (Frederick J Perlak et al., 2001). *Bt* cultivars are environmentally friendly tools for selective pest management and provide a significant economic return in many areas (Gore et al., 2002). Numerous lepidopteran pests such as tobacco budworm, *Heliothis virscens* (F.); *H. zea*, *Helicoverpa zea* (Boddie); and pink bollworm, *Pectinophora gossypiella* (Saunders), are susceptible to the Cry1Ac protein in Bollgard cottons (Frederick J Perlak et al., 2001; Gore et al., 2002; Nathan S Little et al., 2019). *Bt* cotton provides control of *H. zea* , but supplemental insecticide applications have been required periodically for control of this pest since the first commercial deployment of the transgenic plants in 1996 (Randall G Luttrell & Ryan E Jackson, 2012). Transgenic *Bt* cotton can enhance profitability and reduce environmental costs of insect applications, but they have not completely eliminated the need for supplemental foliar insecticide applications against *H. zea* and other lepidopteran pests.

Mode of Action of 3d-Cry toxins in Lepidoptera

Bacillus thuringiensis (*Bt*) insecticidal Cry toxins have been shown to be effective in controlling insect pests either in spray products or expressed in transgenic crops. Identifying a protein as a Cry toxin originates from the fact that it forms a parasporal crystal. Cry toxins do not belong to a single, homologous family of proteins but, instead, include several unrelated lineages (Palma et al., 2014). The most widely used Cry toxins are the 3-domain Cry toxins, so-called based on their common structure but show differences in their amino acid sequences. Cry proteins make up the largest group of insecticidal proteins produced by a species of *Bacillus.* The

Bt Toxin Nomenclature Committee has classified 73 different types of toxins. Examples include Cry1Ac active against certain Lepidoptera, Cry2Ab targeting some Lepidoptera but also active on Diptera, and the coleopteran active Cry3Aa (Heckel, 2020).

The mode of action of 3d-Cry toxins involves sequential interactions with several insect midgut proteins that facilitate the formation of an oligomeric structure and induce its insertion into the membrane, forming a pore that produces the lysis of epithelial cells and hence midgut disarrangements, leading to insect death (Pardo-Lopez et al., 2013). When susceptible larvae ingest the 3d-Cry protoxin, it is solubilized and activated by intestinal proteases, generating a toxic fragment composed of the three-domain structure. The sequential binding model suggests that Cry toxins, once activated by intestinal proteases, bind to cadherin-like proteins that function as toxin receptors (Palma et al., 2014). The activated toxin goes through a complex change leading to membrane insertion and pore formation (Bravo et al., 2004; Pigott & Ellar, 2007; Soberon et al., 2009). Cry toxins form pores in the apical membrane of larvae midgut cells, destroying the cells and killing the larvae (Soberon et al., 2009)

Vip3 Proteins

Vegetative cells produce non-crystalline toxins, such as vegetative insecticidal proteins (Vip), which are secreted as soluble proteins in a growth medium (Syed et al., 2020). The Vip3 proteins do not exhibit any similarity with the Cry toxins (Rang et al., 2005). Currently, there are three subfamilies of Vip3 that include Vip3A, Vip3B, and Vip3C. Vip3A is the most widely studied Vip3 toxin so far and includes a host range of several major lepidopteran insect pests. Much like Cry toxins, Vip3 proteins must be activated by intestinal proteases prior to binding to the midgut epithelial cells. Essentially, Vip3 are like Cry toxins in how it binds to the midgut epithelial cells of the susceptible insects causing their lysis, gut paralysis, and larval death (Rang

et al., 2005). However, it has been suggested that this mode of action differs from that of some Cry1Ac toxins, at least by their receptor binding sites and ion channel properties (Palma et al., 2014). Vip3 proteins make good contenders for resistance management strategies involving stacking or rotating proteins with different insecticidal modes of action (Rang et al., 2005).

Control Strategy for *H. zea*

The threshold for *H. zea* in Arkansas cotton is based upon which cotton cultivar is planted (Studebaker et al., 2023). For conventional cotton the current threshold for *H. zea* in cotton is one *H. zea* less than 6.35 mm per 0.61 m. Two gene cultivar thresholds are reached when there is 6% damaged fruit or 2-3 large (>6.35) larva per 4.3 m. Egg thresholds have been established for *H. zea* in conventional and two-gene cotton cultivars, with a threshold of 25 eggs/ 100 plants (1 egg/ 4 plants). The same larval thresholds are used for three gene cultivars expressing Vip3, with the exclusion that there is no egg threshold for three gene cotton.

The key to effective insecticide applications is to apply directly after egg lay when the larva is small and have not eaten into any fruiting structures yet. Second insecticide applications should not be made at a minimum ten days to utilize any residual available by the applied product. This provides information to determine if the application was considered non-effective, and if potential resistance issues should be addressed.

However, field evolved resistance to *Bt* cry proteins has decreased efficacy of dual gene *Bt* cotton cultivars on *H. zea* (Nathan S. Little et al., 2019; Calvin et al., 2021). Corn and cotton are cultivated within proximity to each other allowing *H. zea* larvae to feed on cotton after corn becomes unsuitable for oviposition. The same or similar *Bt* proteins for lepidopteran control are utilized in both corn and cotton cultivars (Huang, 2021). *Bt* proteins expressed across multiple

crops creates a strong selection pressure on *H. zea* which has resulted in the development of resistance to some *Bt* toxins such as Cry1A (Tabashnik, 2015) and Cry2A (Reisig et al., 2018). The EPA prioritizes insect resistance management (IRM) for Plant – Incorporated Protectants (PIPs), including several *Bt* toxins. To delay resistance, EPA has mandated the implementation of an Insect Resistance Management (IRM) plan for each commercially registered *Bt* PIP. The IRM strategy to counter resistance to *Bt* crops throughout the U.S. depend on three tactics. First, the non-*Bt* refuge strategy which requires growers to plant a proportion of their crop to a non-*Bt* cultivar to serve as a refuge for hosting susceptible insects. *Bt*-susceptible insects would emerge from the non-*Bt* refuge areas and mate with individuals that are potentially resistant homozygous that might emerge from the *Bt* crop (Huang et al., 2011). If occurrence of resistance is low, most offspring will be heterozygous and be killed by the high-dose *Bt* cultivar. Another tactic to delay resistance is to use high-dose *Bt* plants. This is known as a high dose when toxin titers are high enough to kill 95% of individuals heterozygous for a resistant allele (Huang et al., 2011). Pyramiding, another tactic to delay resistance, incorporates multiple *Bt* toxins in the same plant that target a given pest species (Carrière et al., 2015). However, once an insect becomes resistant to one *Bt* toxin component in the pyramid, the effectiveness of the pyramid to delay resistance is reduced because the toxin combination no longer 'redundantly' kills target pests (Carrière et al., 2016). To limit economic crop damage and manage *H. zea* populations, supplemental foliar insecticide applications have increased (Reisig et al., 2018). It is now uncommon for dual gene cotton cultivars to not receive a supplemental insecticide application. Cotton expressing a third insecticidal protein, Vip3, was introduced and stacked with Cry proteins. Three gene cultivars expressing Vip3 appear to offer the greatest level of control of *H. zea* (Kerns et al., 2019) and make good contenders for resistance management strategies. Concerning amounts of selection

pressure is being applied on this toxin and unexpected injury in cultivars expressing Vip3 has been reported (Kerns et al., 2019; Nathan S. Little et al., 2019; Niu et al., 2021).

There is some evidence that, while more efficacious against *H. zea*, three gene cotton cultivars yield less than dual gene cultivars. Despite this yield gap, growers could have greater profits using three gene cultivars due to lower input and production cost. Based on information collected from the Arkansas Field Crops Enterprise Budget, Bollgard II has higher input and production cost than Bollgard 3(Budget, 2022). Compared to Bollgard II cotton, three gene cotton reduces insecticide use, lessens the amount of diesel used, decreases time spent in the field, and has a higher seed cost per acre. Depending on seed cost and yield, the reduction in operating expenses and higher average income could provide the grower with a greater profit margin when planting a three gene cultivar. The objective of this study was to determine if dual or three gene cotton is more cost-effective for growers to plant, with the understanding that the dual gene cotton may need supplemental foliar applications to control *H. zea.*

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Chapter II

Determining efficacy and economic return of *Bt* cotton cultivars and foliar insecticides for control of *Helicoverpa zea*

Abstract

Helicoverpa zea is the second most damaging insect pest of cotton in Arkansas. Foliar insecticides and insecticidal proteins from *Bacillus thuringiensis* (Bt) in transgenic cotton are common *H. zea* management tools used in cotton production. Resistance to dual gene cotton cultivars has recently been documented in *H. zea*, and results indicate that supplemental foliar applications may be needed to manage high populations. Research was conducted in 2021 and 2022 on a grower field in Drew County, Arkansas and repeated in 2022 at the University of Arkansas Lon Mann Cotton Research Station near Marianna, AR, to evaluate the efficacy of several *Bt* technologies and the economic value of dual gene and three gene technologies. Additional research was conducted to evaluate the cost-effectiveness of cheaper short residual insecticides versus more expensive long residual insecticides on non-*Bt*, dual gene, and three gene cotton against *H. zea* were determined. The efficacy and residual control of several insecticides, including chlorantraniliprole, acephate, and bifenthrin were researched. Non-*Bt* plots sprayed with two applications of chlorantraniliprole, and plots sprayed with chlorantraniliprole followed by acephate plus bifenthrin had yields similar to the dual gene and three gene plots. Decreased damage was observed in all plots sprayed with chlorantraniliprole. These data suggest that despite the yield gap, growers could increase profits using three gene cultivars due to lower input and production cost. In recent years the yield gap between two-gene cultivars and three gene cultivars has diminished, and three gene acres have increased from 28%

in 2020 to 89% in 2021. Growers should budget at least one diamide insecticide application when planting anything other than a three gene variety to prevent yield loss.

Introduction

Genetically engineered (GE) crops were rapidly adopted in 1996 when they were commercially introduced in the United States. When growers adopt a new technology, they expect benefits like increased net returns, time savings, and decreased input cost. The widespread adoption of GE seeds indicates farmers have benefited from them. Based on the Agricultural Resource Management Survey, farmers indicate that they adopted GE corn, cotton, and soybeans primarily to increase yields (Fernandez-Cornejo et al., 2014). Throughout the first 15 years of commercial use, GE seeds did not increase yield potentials of the varieties (Fernandez-Cornejo et al., 2014). Yield may even decrease if the varieties used to carry the herbicide-tolerant or insect-resistant genes are not the highest yielding cultivars (Fernandez-Cornejo & Caswell, 2006). This is known as "yield drag" which is the idea that manipulating the genome of a plant variety causes unintended changes in the way it grows causing it to be less productive (Fernandez-Cornejo et al., 2014). However, GE crops prevent yield loss by protecting the plant from certain pest, allowing the plant to approach its yield potential.

The increase in GE seed prices is associated with the rising share of GE seeds with more than one trait and/or more than one mode of action for a particular target pest (NRC, 2010). For example, growers are seeing a seed price increase for Bollgard 3® (Monsanto Co., St. Louis, MO) due to the added protection from the third gene (Vip3a) for the control of *H. zea* when compared to dual gene. Based on the rapid adoption of GE crops, farmers are willing to pay higher seed prices because of improved seed performance, pest management traits, and saving on input cost. Crops genetically engineered to produce insecticidal proteins from *Bacillus*

thuringiensis (*Bt*) can provide safe and effect control of various pests (Abbas, 2018; Romeis et al., 2019). Bollgard® (Monsanto Co., St. Louis, MO) cotton was the first commercial cotton technology that incorporated *Bt* toxins within the crop genetics. Numerous lepidopteran pests such as tobacco budworm, *Heliothis virscens* (F.); *H. zea*, *Helicoverpa zea* (Boddie); and pink *H. zea*, *Pectinophora gossypiella* (Saunders), are susceptible to the Cry1Ac protein in Bollgard cottons (Frederick J. Perlak et al., 2001; Gore, 2002; Nathan S. Little et al., 2019).

Prior to the introduction of transgenic cottons expressing insecticidal proteins derived from *Bt*, foliar insecticides were used as the main control method of *H. zea* (Reisig et al., 2018). Insecticides have a long history of use in cotton that can be divided into three periods. The first period was the DDT and organochlorine period, lasting from their introduction just after World War II to the mid- 1960s when widespread resistance and environmental concerns began (Sparks, 1981). The second period consisted of the organophosphorus insecticides (Sparks, 1981), which is still widely used for control of multiple insect pest in cotton. The use of organophosphorus insecticides declined in the late 1970s when the tobacco budworm developed resistance to various organophosphorus insecticides in use (Sparks, 1981, 1996). Lastly, the third period of pyrethroid insecticides began. Pyrethroids are still frequently used throughout several cropping systems, including cotton, for the control of insect pests. However, pyrethroid resistance is now present across the United States (Luttrell et al., 1987; Leonard et al., 1988; Plapp et al., 1990; Abd-Elghafar et al., 1993). Widespread resistance of multiple insecticides allowed a new era of insect pest control. To avoid the development of resistance in treated pest, new chemistries with activity against *H. zea* were developed during the mid – 1990s through the 2000s (J Gore, 2000; Peters et al., 2003; Herbert Jr et al., 2012). The newer chemistries included insect growth regulators, spinosyns, and diamides which provided control of *H. zea.*

Transgenic *Bt* cotton have not eradicated the need for supplemental foliar insecticidal applications (Fleming et al., 2018). Multiple reports found in the Beltwide Cotton Conference Proceedings *Bt* field efficacy was variable against *H. zea*, with damaged fruit ranging from 1-60%, depending on the density of *H. zea* infestation (Randall G. Luttrell & Ryan E. Jackson, 2012). In 1995, Arkansas growers averaged 4.6 insecticide applications for boll/bud worms (Williams, 1995). Growers treated 95% acres out of the 1 million acres planted in Arkansas with an average application cost of $10.45 (Williams, 1995). In 2021, 100% of the 1,160,900 hectares of cotton planted in Arkansas consisted of transgenic cultivars (Cook, 2021). Approximately 100% of the acres were infested with *H. zea*/Budworm with only 227,536 hectares being treated (Cook, 2021). Growers averaged 1.2 insecticide applications that averaged $31.46 per application (Cook, 2021).

To improve *H. zea* control, second generation pyramided dual gene *Bt* cottons Bollgard II® (Cry 1Ac + Cry 2Ab) and WideStrike™ (Cry1Ac + Cry 1F), both expressing two *Bt* endotoxins, were introduced. Both of these technologies immediately demonstrated improved field efficacy against *H. zea* with reduced larval survival and subsequent fruit damage (Chitkowski, 2003), such that supplemental insecticide sprays did not automatically relate to improved yields (Jackson, 2003; Jackson et al., 2006). However, field studies still occasionally showed that treating dual gene cotton cultivars with pyrethroids for *H. zea* control increased yield (Jackson & Bradley, 2005; Greene et al., 2011). In 2010, supplemental foliar insecticide applications to dual gene cottons became increasingly common (Greene et al., 2011). In 2014 three gene cottons were introduced that express *Bt* derived vegetative insecticidal proteins (Vip3A) that were also stacked with existing Cry proteins. *Bt* cotton cultivars expressing Vip3A genes include Bollgard 3(Cry1Ac+Cry2Ab+Vip3A) and WideStrike3 (Cry1Ac+Cry1F+Vip3A).

The cultivars containing Vip3A appear to offer the greatest level of *H. zea* control to date (Kerns et al., 2019).

Resistance in *H. zea* to *Bt* cotton cultivars has become an important concern due to increased feeding damage on various fruiting forms. Dual gene cotton may not provide the protection needed to manage cotton *H. zea* and foliar applications may be required. There is some evidence that, while more efficacious against *H. zea*, three gene cotton cultivars yield less than dual gene cultivars. Field experiments were conducted to evaluate the efficacy of several *Bt* technologies and determine the economic value of non-*Bt*, dual gene and three gene technologies. Additionally, the cost-effectiveness of cheaper short residual insecticides versus more expensive long residual insecticides on non-*Bt*, dual gene, and Three gene cotton against *H. zea* were determined.

Materials and Methods

Field Experiment Details

Technology Study

Field studies were conducted during 2021 in Tillar, Arkansas and repeated in 2022 at both the University of Arkansas Lon Mann Cotton Branch Experiment Station near Marianna, AR and in Tillar, AR. Plots were planted using a non-*Bt* (DP 1822 XF, Bayer CropScience, St. Louis, Mo), a dual gene (DP 1518 B2XF, Bayer CropScience, St. Louis, Mo), and two three gene cultivars (DP 1845 B3XF, Bayer CropScience, St. Louis, Mo), (PHY 400 W3FE, Corteva Agriscience, Indianapolis, Indiana). All trials were planted in a randomized complete block design with 4 replications with a John Deere Max Emerge 2 7300, four row planter modified with Almaco 31 cell cones for plot planting. Each variety was planted at 135,850 seed ha^{-1} into

conventionally tilled raised beds between 16 May and 18 May in 2021 and 2022. Plot size was four rows, 96.5 cm apart and 12.2 m long. Fallow alleys measuring 3.04 m long separated the replications. The soils at these sites are Memphis silt loam (fine-silty, mixed, active, thermic Typic Hapludalfs) and Rilla silt loam (fine-silty, mixed, active, thermic Typic Hapludalfs) at Marianna and Tillar, respectively. All trials were conducted under furrow irrigated production practices at both locations. Weeds were controlled throughout the growing season with herbicides and manual removal of weeds. Plots were over sprayed with sulfoxaflor (Transform, Corteva Agriscience, Indianapolis, Indiana) every 7 to 10 days to reduce plant bug population (Studebaker et al., 2023). Plots were managed using standard production practices recommended by the University of Arkansas Cooperative Extension Service.

Each cultivar had a plot that either remained unsprayed or was sprayed with 96 (g ai/ha) of chlorantraniliprole (Prevathon 0.43 SC, FMC Corporation; Philadelphia, PA) for a total of 8 treatments. The chlorantraniliprole application was made when the University of Arkansas Cooperative Extension Service threshold of 20% egg lay was met (Studebaker et al., 2023). All pesticides were applied using a Mudmaster high clearance sprayer fitted with TXVS-6 flat fan nozzles at 50 cm. spacing with a spray volume of 94 L/ha, at 276 kPa. chlorantraniliprole was applied on 22 July at the Tillar location in 2021 and 19 Jul and 27 Jul at the Tillar and Marianna locations in 2022, respectively. Data collection occurred 3, 7, 10, 14, and 21 days after application (DAA). In each plot, 25 squares, 25 flowers, and 25 bolls were sampled, and the number damaged for each was recorded. The two center rows of each plot were harvested on 1 Nov at the Tillar location in 2021 and 13 Oct and 1 Nov at the Tillar and Marianna locations in 2022, respectively. Yield was reported as kg/ha of seed cotton. All data were processed using PROC GLIMMIX in SAS v 9.4 (SAS Institute, Inc., Cary N.C.) with $\alpha = 0.05$.

Foliar Trial

Field studies were conducted during 2021 in Tillar, Arkansas and repeated in 2022 at both the University of Arkansas Lon Mann Cotton Branch Experiment Station near Marianna, AR and in Tillar, AR. Plots were planted using a non-*Bt* (DP 1822 XF, Bayer CropScience, St. Louis, Mo), a dual gene (DP 1518 B2XF, Bayer CropScience, St. Louis, Mo), and a Three gene cultivar (DP 1845 B3XF, Bayer CropScience, St. Louis, Mo). Each variety was planted at 135,850 seed ha^{-1} into conventionally tilled raised beds between 16 May and 18 May in 2021 and 2022. Plot size was four rows, 96.5 cm apart and 12.2 m long. Fallow alleys measuring 3.04 m long separated the replications. Plots were over sprayed with sulfoxaflor (Transform, Corteva Agriscience, Indianapolis, Indiana) every 7 to 10 days to reduce plant bug population (Studebaker et al., 2023). Treatments within each cultivar included: Untreated Check (UTC); chlorantraniliprole (96 g ai/ha (Prevathon, FMC Corporation; Philadelphia, PA); chlorantraniliprole (96 g ai/ha) followed by chlorantraniliprole (96 g ai/ha); chlorantraniliprole (96 g ai/ha) followed by acephate (168 g ai/ha) (Orthene 97, AMVAC Chemical Corporation: Newport Beach, CA) plus bifenthrin (148 g ai/ha) (Bifen I/T, Control Solutions Inc: Pasadena, TX); and acephate (168 g ai/ha) plus bifenthrin (148 g/ha). The first applications of chlorantraniliprole and acephate plus bifenthrin were applied on 22 Jul at the Tillar location in 2021 and 19 Jul and 27 Jul at the Tillar and Marianna locations in 2022, respectively. Data collection occurred 3,7, 14, and 20 days after application (DAA1) for the first series of sprays. For the plots receiving a second application of chlorantraniliprole and acephate plus bifenthrin, applications were made on 9 Aug at the Tillar location in 2021 and 4 Aug at the Tillar location in 2022, respectively.

For the two plots within each technology, one received an additional application of chlorantraniliprole and the other received acephate plus bifenthrin. Data collection occurred 3, 8, 12, and 20 days after application (DAA2). Each application was made using a Mudmaster high clearance sprayer fitted with TXVS-6 flat fan nozzles at 50 cm. spacing with a spray volume of 94 L/ha, at 276 kPa. In each plot, 25 squares, 25 flowers, and 25 bolls were sampled, and the number damaged for each was recorded. The two center rows of each plot were harvested on 1 Nov at the Tillar location in 2021 and 13 Oct and 1 Nov at the Tillar and Marianna locations in 2022, respectively. Yield was reported as kg/ha of seed cotton. All data were processed using PROC GLIMMIX in SAS v 9.4 (SAS Institute, Inc., Cary N.C.) with $\alpha = 0.05$.

Results

Interactions were tested for. However, due to no interactions and significant differences in *H. zea* populations over the 2021 and 2022 year, it was broken up by year.

Sprayed Vs Unsprayed Results

In 2021, the initial observation (4 d), unsprayed non-*Bt* plots contained the greatest amount of damaged fruit at 23% (Table 1). Sprayed non-*Bt*, sprayed dual gene, and all three gene plots had the least amount of damage. At seven days, the percent damaged fruit was greatest in unsprayed non-*Bt* and unsprayed dual gene (Table 1). Three gene sprayed non-*Bt* and sprayed dual gene plots had lesser amounts of damage than the previously mentioned plots. Similar amounts of damage were observed in unsprayed non-*Bt* and unsprayed dual gene throughout the growing season. Damage levels observed at ten and fourteen days after application are analogous to those found seven days after application. All three gene plots, sprayed dual gene and sprayed non-*Bt* plots, contained a lesser amount of damage than unsprayed non-*Bt* and unsprayed dual

gene plots (Table 1). At twenty-one days after application, the total damaged fruit levels decreased. *H. zea* larvae were observed transitioning to the pupae stage across plots. Both unsprayed non-*Bt* and unsprayed Bollgard II had significantly lower yields (Table 2). Unsprayed non-*Bt* and Unsprayed Bollgard II benefited from a chlorantraniliprole application.

Seasonal fruit damage was relatively low at the Tillar and Marianna locations in 2022. Unsprayed non-*Bt* fruit damage in both locations four days after application were 22.6% and 14%, respectfully, and was the only cultivar to benefit from the diamide application (Table 1). Percent damage was reduced in the following observations made in Marianna, AR (Table 1). However, in Tillar at ten and fourteen days, damage in the unsprayed non-*Bt*, unsprayed dual gene, and Unsprayed three gene cultivars was above the 6% damaged fruit threshold. Yields in Tillar showed no significant difference between any treatments (Table 2). In the Marianna location, the yields for both unsprayed non-*Bt* and unsprayed Bollgard II were lower than all other treatments (Table 2).

Foliar Results

In 2021, at 4 DAA, the unsprayed non-*Bt* plots had the greatest amount of damaged fruit at 9 percent (Table 3). Both the sprayed dual gene and three gene plots had similar amounts of damage and had less than unsprayed non-*Bt*. At 7 DAA, the percent damaged fruit was greatest in unsprayed non-*Bt* (Table 3). Insecticides across each technology, except acephate plus bifenthrin in the non-*Bt*, adequately reduced fruit damage. Similar trends were observed for the 10 DAA sampling time. Decreased damage was noticed across all plots at 14 DAA possibly due to *H. zea* larvae transitioning to pupae stage. During data collection at 21 DAA1, all non-*Bt* plots and unsprayed dual gene were above the 6% fruit damage threshold, which initiated the second insecticide application. The second application consisted of a second chlorantraniliprole

application or an application of acephate plus bifenthrin. Decreased damage was noticed across all plots that received two applications of insecticide except the non-*Bt* plots receiving the second application (Table 3). All plots that received a second application of insecticide had similar levels of damage and had less damage than plots that did not receive a second application, except all three gene plots. Non-*Bt* plots sprayed with two applications of chlorantraniliprole, and plots sprayed with chlorantraniliprole followed by acephate plus bifenthrin had yields similar to the dual gene and three gene plots (Table 4).

In 2022, plots located at Tillar, Arkansas differed from plots located in Marianna, Arkansas due to *H. zea* population (Table 3). In Tillar, Similar observations can be made from three days after application to fourteen days after application in non-*Bt* plots both sprayed and unsprayed. The percent damaged fruit did not get below the 6% fruit damage threshold in any of the non-*Bt* plots between three and fourteen days after application. When the second application was initiated, decreased damage was observed in all plots receiving the second application. Each plot that received a second application of insecticide had similar levels of damage and had less damage than plots that did not receive a second application, except all three gene plots (Table 3).

In Marianna at 7 DAA the unsprayed non-*Bt* plots had the greatest amount of damaged fruit at 24 % (Table 3) Both the dual gene and three gene plots had similar amounts of damage and had less than unsprayed non-*Bt*. At 10 DAA, the percent damaged fruit was greatest in unsprayed non-*Bt.* Insecticides across each technology, except acephate plus bifenthrin in the non-*Bt*, adequately reduced fruit damage but did not get the non-*Bt* plots below the 6% fruit damage threshold. Similar trends were observed for the 14 DAA sampling time. Decreased damage was noticed across all plots at 21 DAA due to cotton reaching cutout and a low *H. zea* population. The second foliar insecticide application was not triggered. In the Marianna and

Tillar locations in 2022, Non-*Bt* plots sprayed with two applications of chlorantraniliprole, and plots sprayed with chlorantraniliprole followed by acephate plus bifenthrin had yields similar to the dual gene and three gene plots (Table 4).

Profit and Breakeven

In 2021, growers planting Bollgard II cotton lost $654 per hectare and broke even 0.16% of the time based on simulated price and cost per acre (Table 5). When Bollgard II was sprayed with chlorantraniliprole, profit increased to $253 per hectare and breakeven percentage increased to 65% (Table 5). In 2022, across both sites, lower *H. zea* pressure allowed Bollgard II to provide adequate protection without a foliar insecticide application (Table 6-7). When planting Bollgard 3, growers are breaking even >70% of the time, with average profit of $403 per hectare. In 2021, the simulated foliar trial showed a loss per acre across all Bollgard II treatments except when sprayed with chlorantraniliprole and followed by acephate + bifenthrin when threshold was met a second time (Table 8). Increased yield were seen in 2022 across all foliar treatments which increased the overall profit and breakeven percentage for growers (Table 8).

A shift in technology acreage has been observed over recent years. In 2017 and 2018 the predominate cotton acreage was planted with dual gene cultivars in the state of Arkansas (Table 9). However, since 2019 growers have started to shift acreage to three gene cultivars, nearly flipping the technology planted in three short years (Table 9). Three gene cultivars are being planted across more acres due to higher yields compared to dual gene cultivars (Table 9). Growers planting three gene cultivars have noticed reduced input cost when compared to two gene cultivars (Table 10).

Discussion

Overall, in 2022, *H. zea* infestation pressure was low compared to previous years despite *H. zea* pheromone traps showing high populations. Seasonal fruit damage was relatively low at the Tillar and Marianna locations. Due to the cost of *Bt* technology and increased need for supplemental control with foliar insecticide sprays, dual gene cotton may no longer be an economical option for *H. zea* control (Reisig et al., 2018). Based on findings from this experiment, dual gene appears to have lost much of its efficacy to prevent fruit damage from *H. zea*. Dual gene alone may not provide satisfactory control of *H. zea* when pressure is moderate or greater and supplemental foliar insecticide applications may be required. Three gene provides exceptional control and decreases the amount of supplemental foliar applications required for *H. zea*. Grower's planting dual gene cultivars should budget at least one application of a diamide insecticide to prevent yield loss. Based on information collected from the Arkansas Field Crops Enterprise Budget, dual gene has higher input and production cost than three gene (Table 10) (Crop Enterprise Budget 2023). Compared to dual gene cotton, three gene cotton reduces insecticide use, lessens the amount of diesel used, decreases time spent in the field, and has a higher seed cost per acre (Table 10). Yields were compared between the dual and three gene cultivars from data generated by the On-Farm Variety Trials (OVT) conducted in Arkansas (Bourland et al., 2020). Three gene cultivars have recently started to yield higher but are closely followed by dual gene cultivars (Table 9). However, three gene cultivars ($715) provided a $115 per acre advantage compared to dual gene cotton ($600). Depending on seed cost and yield, the reduction in operating expenses and higher average income could provide the grower with a greater profit margin when planting a three gene cultivar.

Resistance to the Cry proteins in cotton has increased in recent years (Fleming et al., 2018). Increased damage and yield reductions are observable in unsprayed dual gene cotton cultivars (Fleming et al., 2018; Reisig et al., 2018) compared to previous years. This has led to an increasing number of foliar insecticide sprays to manage *H. zea* populations and limit economic crop damage (Hardee et al., 2001). In a previous study performed by Fleming et al., (2018), dual gene reduced damage relative to non-*Bt* by 81%. Population density, host plant abundance, and environmental factors appear to influence *H. zea* feeding on *Bt* cotton. Based on findings of the current study, supplemental foliar application may be required to manage high populations of cotton *H. zea*. Repeated applications of insecticides for the control of *H. zea* has led to documented resistance in carbamates, organophosphates, and pyrethroids (Stadelbacher et al., 1990), causing these compounds to no longer provide adequate control. Once diamides were released in 2008 (EPA, 2008), they became the leading lepidopteran insect pest control agent across multiple cropping systems. Despite being highly efficacious for control of *H. zea* , they are expensive compared to other classes of insecticides (Little et al., 2017). Now, diamides or combinations of pyrethroids and organophosphates are used in supplemental control of *H. zea* when populations exceed threshold levels in *Bt* cotton throughout the Mid-South United States.

The widespread resistance in *H. zea* to multiple cry proteins is creating a dependency on Vip3Atechnology to provide sufficient for *H. zea*. Similar to Bollgard and dual gene, high selective pressure can lead to technology becoming less susceptible. Variable tissue expression and single-gene dependency for control will likely minimize the amount of time Vip3A will provide high mortality for Cry-resistant *H. zea* populations (Godbold, 2020).

Introduction of new *Bt* protein have increased the efficacy of transgenic cotton cultivars targeting lepidopteran pest, including *H. zea*. In recent years unexpected *H. zea* damage has

become common place in dual gene *Bt* cotton and insecticide applications targeting *H. zea* have been necessary to avoid unacceptable injury and yield loss (Kerns, 2022). Despite increased supplemental foliar insecticide sprays to manage populations of *H. zea* in dual gene, it is geographically variable, depending on *H. zea* pressure and climate. In a recent study test were conducted across seven locations throughout the Mid-South and one location in Texas to determine the efficacy of *Bt* cotton technologies and if an insecticide treatment results in higher yields (Kerns, 2022). *Bt* cotton technologies evaluated included: WideStrike 3, TwinLink Plus, Bollgard II, and Bollgard 3. A non-*Bt* variety was included as a check. Across seven of the eight locations, seasonal fruit damage did not differ from any of the *Bt* traits. However, the seasonal percent fruit damage for dual gene did not differ from the non-*Bt*. Dual gene only failed to offer significant *H. zea* protection at this location.

Overall, *H. zea* infestation pressure was low compared to previous years. This might be due to high amounts of rainfall or abnormally cold temperatures. Based on findings from this experiment, dual gene appears to provide adequate control of *H. zea* under low pressure. However, dual gene alone may not provide satisfactory control of *H. zea* when pressure is moderate or greater and supplemental foliar insecticide applications may be required. Three gene cultivars appear to provide sufficient control of *H. zea* but should still be monitored to prevent yield loss. Growers applying chlorantraniliprole will achieve adequate control in two-gene cultivars. Due to recently documented resistance in *H. zea* to multiple cry proteins, growers should budget at least one diamide insecticide application when planting anything other than a three gene variety to prevent yield loss. When selecting cultivars, yield potential should be considered first and then technology.

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Table 1. Percent total damage of combined fruiting structures recorded at 4,7,10,14, and 21 days after a chlorantraniliprole application using four cotton cultivars during 2021-2022 growing seasons.

Treatments	Tillar 2021					Tillar 2022					Marianna 2022				
	4	**7**	**10**	**14**	**21**	**4**	**7**	**10**	**14**	**21**	**4**	**7**	**10**	**14**	**21**
Non-Bt	23a	24a	37a	34.3a	13a	22.6a	4abc	17.5a	15a	14.5ab	14a	25a	31.9a	25a	17a
Bollgard II	12b	24a	33.6a	23.6b	7b	7.2bc	3.3abc	11.7ab	17.3a	3.5ab	2.6b	0.3b	1.3bc	4b	5b
Bollgard 3	5.7bc	1b	0.3b	1c	0c	0.25c	10.2a	17.2a	25a	26a	0b	0b	0c	0d	0c
WideStrike 3	3.7c	0.6b	2b	0.6c	0.3c	1c	8.5ab	17.25a	17.3a	8ab	0.6b	0b	0c	1bcd	0c
Non-Bt*	3.7c	0.6b	1.3b	0c	0.6c	12.5ab	1.5bc	1.25b	1.3a	2ab	10.3a	10.3a	9.3b	3.5bc	2bc
Bollgard II*	5.5bc	2b	0.3b	0.6c	0.6c	0c	2.2bc	2.5ab	0a	2ab	0.3b	0b	0c	0.5cd	1bc
Bollgard 3*	2.2c	1b	0.3b	0c	0.6c	0.25c	0.2c	2.25ab	1a	1.5ab	0.3b	0.3b	0c	0d	1bc
Widestrike 3*	2.2c	1.6b	0.6b	2.3c	0c	0.5c	1.5bc	1b	5a	0.5ab	0.6b	0.3b	0c	0d	2bc
Num df	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Den df	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
F	9.5	7.35	23.5	16.5	7.9	5.8	1.8	2.1	1.1	1.1	10.6	10.6	14.8	64.2	13.8
P	<.0001	0.0002	<.0001	<.0001	<.0001	0.0005	0.145	0.0809	0.3917	0.422	<.0001	<.0001	<.0001	<.0001	<.0001

Average percent total damage followed by a different letter are significantly different according to a pairwise t-test at an α=0.05
* Sprayed with chlorantraniliprole

Table 2. Average lint yield expressed in kg per hectare across four cotton cultivars with and without a diamide application.

Treatments	Tillar 2021 Mean yield (kg/ha)	Tillar 2022 Mean yield (kg/ha)	Marianna 2022 Mean yield (kg/ha)
Non-Bt	374.1b	1371.7a	749.3e
Bollgard II	244.9b	1390.8a	1096.9d
Bollgard 3	1276.5a	1665.9a	1199.9cd
WideStrike 3	1515.6a	1558.8a	1239cd
Non-Bt*	1392.2a	1493.4a	1376.5abc
Bollgard II*	1434.6a	981.4a	1478.1ab
Bollgard 3*	1467.4a	1381.2a	1289.7bcd
Widestrike 3*	1291.9a	1255.1a	1537.5a
Num df	7	7	7
Den df	21	21	21
F	8.17	0.74	9.92
P	<.0001	0.6376	<.0001

Average lint yield followed by a different letter are significantly different according to a pairwise t-test at an α=0.05

* Sprayed with chlorantraniliprole

Table 3. Percent total damage of combined fruiting structures recorded at 4,7,10,14, 21, and 28 days after a foliar insecticide application using three cotton cultivars during 2021-2022 growing seasons.

Treatment	Tillar 2021						Tillar 2022						Marianna 2022			
DAT	**4**	**7**	**10**	**14**	**21**	**28**	**4**	**7**	**10**	**14**	**21**	**28**	**7**	**10**	**14**	**21**
Non-Bt	9.0a	16.7a	40.3a	27.33a	22.00a	48.a	19.7a	31.2a	41.7a	58.0a	62.3a	17a	23.7a	30.7a	23.0a	17.0a
Chl*	2.0bc	0.33d	2.3bc	1.33bc	5.83cd	26.3c	11.7c	13.7b	20.2c	12.6b	1.5	3bc	8.0c	7.7c	7.5c	2.0c
Chl fb Chl"	2.3bc	0.67d	2.3bc	2.0bc	7.50c	15.9d	18.2ab	17b	19.7c	16.3b	2b	0c	3.7d	6.0cd	9.0c	2.0c
Chl fb Ace+Bif"	2.33bc	0.67d	1.34c	0.00c	6.2cd	15.8d	11c	21.2b	18.5c	40.6a	1b	1bc	8.3c	6.0cd	6.0cd	3.0c
Ace +Bif*	1.67bc	4.00b	9.00b	1.00c	13.2b	35.71b	13bc	18.5b	26.2b	17.3b	13b	14a	13.0b	19.7b	14.5b	10.0b
BGII	3.67b	2.34c	3.7bc	4.33b	6.83c	12.33d	4d	2.2c	2.5d	7.6b	6b	5b	2.0d	1.7de	4.5cde	12.0ab
Chl*	2.00bc	0.33d	0.67c	0.33c	2.50de	5.50e	0.25d	0.5c	0.5d	0.33b	1.5b	2bc	0.0d	0.7e	1.0de	0.0c
Chl fb Chl"	0.33c	0.00d	0.67c	0.33c	0.83e	3.38e	2.5d	1.7c	1.5d	1.3b	0b	0c	0.0d	0.0e	1.5de	1.0c
Chlfb Ace+Bif"	1.7bc	0.00d	1.33c	0.67c	2.67e	3.50e	1.5d	1.2c	1d	2.6b	0.5b	0c	0.3d	0.3e	1.5de	3.0c
Ace +Bif*	0.33c	0.00d	1.34c	1.33bc	2.67e	12.25d	1.5d	2c	0.5d	2b	2b	5b	1.3d	0.7e	0.5e	1.0c
BG3	0.67c	0.33d	0.00c	0.00c	0.50e	0.00e	3.75d	0c	0.5d	0.33b	0b	0c	0.3d	0.0e	0.0e	0.0c
Chl*	0.67c	0.33d	0.33c	0.67c	0.67e	0.17e	1.25d	0.5c	0.25d	0b	0b	0c	0.0d	0.0e	0.0e	0.0c
Chl fb Chl"	0.33c	0.00d	0.33c	0.00c	0.00e	0.00e	1.7d	0.75c	0.25d	0b	0b	0c	0.0d	0.0e	0.0e	0.0c
Chl fb Ace+Bif"	0.33c	0.00d	0.00c	0.33c	0.83e	0.00e	1.5d	0.5c	0.25d	0b	0b	0c	0.7d	0.0e	0.0e	0.0c
Ace +Bif*	0.67c	0.00d	0.00c	0.00c	0.33e	0.50e	0.5d	0c	0d	1.6b	0b	0c	1.0d	0.0e	0.0e	0.0c
Num Df	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
Den DF	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42
F	5.2	84.3	18.9	41.8	17.9	36.5	12.5	12.2	49.1	6.7	7.7	11.9	20.7	26.4	13.1	8.9
P	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Percent total damage followed by a different letter are significantly different according to a pairwise t-test at an α=0.05
Chl = Chlorantraniliprole (96 g ai/ha) | Ace = Acephate (168 g ai/ha) | Bif = Bifenthrin (148 g ai/ha)
Second application was triggered at 28 DAA in Tillar 2021 and 2022. It was not triggered in Marianna 2022 due to cotton reaching cutout.
Day 28 was omitted from the 2022 Marianna location due to cotton reaching cutout.
*One insecticide application
" Plots received two insecticide applications

Table 4. Average lint yield expressed in kg per hectare across three cotton cultivars with and without a foliar insecticide application.

Location	Treatment	Non-Bt	BG II	BG 3	Stats
Tillar 2021	UNT	2101.7ab	2227ab	3249a	Num Df 14 Den Df 42 F = 1.07 *P* = 0.412
	Chl*	2901.5ab	1745b	2709.1ab	
	Chl fb Chl"	2858.5ab	2101.7ab	3157.4a	
	Chl fb Ace+Bif"	2694.6ab	3104.4a	2492.2ab	
	Ace +Bif*	2540.4ab	2497ab	2921.2ab	
Tillar 2022	UNT	724.8e	1777.4abc	1705.5abcd	Num Df 14 Den Df 42 F = 4 *P* = 0.002
	Chl*	1364.7cd	1935.5ab	1648.8abcd	
	Chl fb Chl"	1794.2abc	2040.4a	1689.8abcd	
	Chlfb Ace+Bif"	1514.8bcd	1753.9abc	1794.7abc	
	Ace +Bif*	1254.6d	1579.9bcd	1709.1abc	
Marianna 2022	UNT	542.6f	1335.09bcd	1201.86cde	Num Df 14 Den Df 42 F = 5.4 *P* = <.001
	Chl*	1039.69de	1666.36a	1170.81cde	
	Chl fb Chl"	1290.75bcde	1572.46ab	1162.13cde	
	Chl fb Ace+Bif"	1084.81cde	1353.41abdc	1244.85cde	
	Ace +Bif*	984.55e	1177.95cde	1378.87abc	

Average lint yield followed by a different letter are significantly different according to a pairwise t-test at an α=0.05
Chl = Chlorantraniliprole (96 g ai/ha) | Ace = Acephate (168 g ai/ha) | Bif = Bifenthrin (148 g ai/ha)
*One insecticide application
" Plots received two insecticide applications

Table 5. Economic Chance and Return of *Bt* technology with and without a chlorantraniliprole application based on average yield using simulated profit and breakeven percentage in Tillar, AR 2021.

Treatment	Avg Yield (kg/ha)	Sim Price†	Sim Cost†	Profit	Breakeven %
Non-Bt	374.1	0.94	N/A	N/A	N/A
Bollgard II	244.9	0.94	860.47	-654.86	0.16%
Bollgard 3	1276.5	0.94	822.96	449.55	94.19%
WideStrike 3	1515.6	0.94	822.96	248.79	67.70%
Non-Bt*	1392.2	0.94	N/A	N/A	N/A
Bollgard II*	1434.6	0.94	880.47*	253.61	65.17%
Bollgard 3*	1467.4	0.94	842.96*	241.75	74.06%
Widestrike 3*	1291.9	0.94	842.96*	389.07	93.44%

† Simulated price represents the five year average cotton market price
† Simulated cost represents the average expenses per acre based on the Arkansas Field Crops Enterprise budget
* Sprayed with chlorantraniliprole
*Add $20 to simulated cost to account for cost of chlorantraniliprole application.

Table 6. Economic Chance and Return of Bt technology with and without a chlorantraniliprole application based on average yield using simulated profit and breakeven percentage in Tillar, AR 2022.

Treatment	Avg Yield (kg/ha)	Sim Price†	Sim Cost†	Profit	Breakeven %
Non-Bt	1371.7	0.94	N/A	N/A	N/A
Bollgard II	1390.8	0.94	860.47	307.29	71.35%
Bollgard 3	1665.9	0.94	822.95	575.82	92.09%
WideStrike 3	1558.8	0.94	822.95	485.81	89.13%
Non-Bt*	1493.4	0.94	N/A	N/A	N/A
Bollgard II*	981.5	0.94	880.47*	56.41	43.54%
Bollgard 3*	1381.2	0.94	842.95*	316.70	74.30%
Widestrike 3*	1255.1	0.94	842.95*	210.82	59.33%

† Simulated price represents the five year average cotton market price
† Simulated cost represents the average expenses per acre based on the Arkansas Field Crops Enterprise budget
* Sprayed with chlorantraniliprole
*Add $20 to simulated cost to account for cost of chlorantraniliprole application.

Table 7. Economic Chance and Return of Bt technology with and without a chlorantraniliprole application based on average yield using simulated profit and breakeven percentage in Marianna, AR 2022

Treatment	Avg Yield (kg/ha)	Sim Price†	Sim Cost†	Profit	Breakeven %
Non-Bt	749.3	0.94	N/A	N/A	N/A
Bollgard II	1096.9	0.94	860.47	60.56	59.57%
Bollgard 3	1199.9	0.94	822.95	184.53	72.59%
WideStrike 3	1239.1	0.94	822.95	197.39	77.75%
Non-Bt*	1376.5	0.94	N/A	N/A	N/A
Bollgard II*	1478.2	0.94	880.47*	360.63	92.75%
Bollgard 3*	1289.8	0.94	842.95*	239.97	77.95%
Widestrike 3*	1537.6	0.94	842.95*	428.01	95.47%

† Simulated price represents the five year average cotton market price
† Simulated cost represents the average expenses per acre based on the Arkansas Field Crops Enterprise budget
* Sprayed with chlorantraniliprole
*Add $20 to simulated cost to account for cost of chlorantraniliprole application.

Table 8. Economic Chance and Return of Bt technology with and without a foliar insecticide application based on average yield using simulated profit and breakeven percentage during 2021-2022 growing seasons.

Location	Treatment	BG II					BG 3				
		Avg Yield (kg/ha)	Sim Price†	Sim Cost†	Profit	Breakeven %	Avg Yield (kg/ha)	Sim Price†	Sim Cost†	Profit	Breakeven %
Tillar 2021	UNT	1962.193	0.94	860.47	-112.50	35.86%	2862.582	0.94	822.95	268.22	82.08%
	Chl*	1537.476	0.94	880.47^	-294.40	15.72%	2386.909	0.94	842.95	66.90	56.03%
	Chl fb Chl”	1851.759	0.94	900.47+	-194.60	24.13%	2781.887	0.94	862.95	197.46	69.32%
	Chl fb Ace+Bif”	2735.179	0.94	887.07’	1042.61	100.00%	2195.781	0.94	849.55	-12.55	47.09%
	Ace +Bif*	2200.029	0.94	867.07#	-28.44	43.51%	2573.789	0.94	829.55	151.53	59.14%
Tillar 2022	UNT	3915.049	0.94	860.47	631.89	89.46%	3756.623	0.94	822.95	609.01	94.48%
	Chl*	4263.294	0.94	880.47^	744.64	95.14%	3631.74	0.94	842.95	541.42	96.42%
	Chl fb Chl”	4494.338	0.94	900.47+	812.72	97.49%	3722.216	0.94	862.95	555.90	96.51%
	Chlfb Ace+Bif”	3863.228	0.94	887.07’	585.54	92.33%	3953.26	0.94	849.55	657.37	97.36%
	Ace +Bif*	3480.131	0.94	867.07#	459.51	93.25%	3764.675	0.94	829.55	605.49	94.64%
Marianna 2022	UNT	2940.733	0.94	860.47	260.50	79.30%	2647.247	0.94	822.95	186.14	73.31%
	Chl*	3670.395	0.94	880.47^	518.64	94.67%	2578.878	0.94	842.95	140.07	69.47%
	Chl fb Chl”	3463.558	0.94	900.47+	419.79	91.71%	2559.76	0.94	862.95	112.79	66.45%
	Chl fb Ace+Bif”	2981.092	0.94	887.07’	249.28	78.55%	2741.972	0.94	849.55	196.24	69.83%
	Ace +Bif*	2594.587	0.94	867.07#	121.95	64.05%	3037.137	0.94	829.55	328.16	88.26%

† Simulated price represents the five year average cotton market price
† Simulated cost represents the average expenses per acre based on the Arkansas Field Crops Enterprise budget
*One insecticide application
“ Plots received two insecticide applications

^ Added $20 to simulated cost to represent cost of chlorantraniliprole application
+ Added $40 to simulated cost to represent cost of two chlorantraniliprole applications
# Added $6.60 to simulated cost to represent cost of acephate+bifenthrin application
‘Added $26.60 to simulated cost to represent cost of chlorantraniliprole, acephate, and bifenthrin application

Table 9. Average yields compared between the dual and three gene cultivars from data generated by the On-Farm Variety Trials (OVT) conducted in Arkansas and percent of cotton technology acreage in Arkansas over the past five years.

Technology		2017	2018	2019	2020	2021
Dual gene		DP 1646 B2XF PHY 444 WRF	DP 1725 B2XF DP1646 B2XF	DP 1725 B2XF DG 3385 B2XF	DP 1725 B2XF NG 3729 B2XF	DP 1646 B2XF DP 1518 B2XF
Three gene		PX4A57 W3FE PX4A54 W3FE	PHY 430 W3FE PHY 340 W3FE	PHY 400 W3FE DP 2012 B3XF	DP 2115 B3XF NG 3195 B3XF	ST 5091 B3XF DP 2127 B3XF
Lint Yield kg/ha		3,497 3,417	3,935 3,516	3,418 3,668	3,488 3,747	3,689 3,908
%	Dual Gene	97%	96%	81.5%	71%	11.1%
Hectares	Three Gene	1%	2.5%	17%	28%	88.9%

Table 10. Main operating expenses for growers planting dual and three gene cotton cultivars taken from the Arkansas Field Crops Enterprise Budget 2023

The four main operating expenses for growers planting Bollgard II and Bollgard 3 cotton taken from the Arkansas Field Crops Enterprise Budget 2023

Operating Expenses	Unit	Quantity		Price/Unit		Cost	
		BG II	BG 3	BG II	BG 3	BG II	BG 3
Insecticide	**Hectare**	2.47	2.47	$73.00	$52.00	$73.00	$52.00
Diesel (Pre/Post Harvest)	**Gallons**	5.42	5.29	$4.50	$4.50	$24.40	$23.82
Labor, Field Activities	**Hrs**	0.929	0.915	$12.45	$12.45	$11.57	$11.39
Seed, Per Acre	**Thous**	117,325	117,325	$2.47	$3.01	$117.33	$142.98

Table 11. Mean percent damage of cotton squares recorded at 4,7,10,14, and 21 days after a chlorantraniliprole application using four cotton cultivars during 2021-2022 growing seasons.

Treatments	Tillar 2021					Tillar 2022				Marianna 2022		
	4	**7**	**10**	**14**	**21**	**4**	**7**	**10**	**14**	**4**	**7**	**10**
Non-Bt	24a	27a	49a	57a	17a	26.4a	7.3ab	20ab	25a	19a	24a	54a
Bollgard II	12b	41 s	51a	39b	12a	8.8bc	5.9ab	13ab	19a	1b	1b	4b
Bollgard 3	7bc	0b	0b	1c	0b	0c	0b	19ab	25a	0b	0b	0b
WideStrike 3	8bc	0b	3b	1c	0b	1c	12ab	27a	21a	0b	0b	0b
Non-Bt*	4bc	0b	2b	0c	1b	16ab	2b	2	0a	15a	3b	9b
Bollgard II*	3bc	1b	0b	2c	0b	0c	4b	3b	0a	0b	0b	0b
Bollgard 3*	2c	2b	0b	0c	1b	0c	0b	3b	0a	0b	0b	0b
Widestrike 3*	2c	2b	0b	3c	0b	0c	1b	2b	7a	0b	0b	0b
Num df	7	7	7	7	7	7	7	7	7	7	7	7
Den df	21	21	21	21	21	21	21	21	21	21	21	21
F	5.7	7.1	45	17.3	8.9	5.5	1.84	1.8	1.36	5.5	30.9	35.9
P	0.0008	0.0002	<.0001	<.0001	<.0001	0.0008	0.1367	0.1255	0.273	0.001	<.0001	<.0001

Average percent square damage followed by a different letter are significantly different according to a pairwise t-test at an α=0.05
Day 14 in Marianna 2022 and day 21 in Tillar and Marianna 2022 were omitted due to zero squares on the plant.
* Sprayed with chlorantraniliprole

Table 12. Mean percent damage of cotton blooms recorded at 4,7,10,14, and 21 days after a chlorantraniliprole application using four cotton cultivars during 2021-2022 growing seasons.

Treatments	Tillar 2021					Tillar 2022					Marianna 2022			
	4	**7**	**10**	**14**	**21**	**4**	**7**	**10**	**14**	**21**	**4**	**7**	**10**	**14**
Non-Bt	16a	25a	30a	19a	7a	20a	2.6a	10abc	5a	4ab	11a	28a	36a	22a
Bollgard II	8b	18a	23a	13b	2b	8.8ab	0a	14a	21a	2ab	4bc	0b	2c	4bc
Bollgard 3	4b	2b	1b	1c	0b	1b	6a	15a	25a	26a	0c	0b	0c	0c
WideStrike 3	3b	1b	1b	1c	0b	2b	7a	13ab	16a	7ab	0c	0b	0c	1bc
Non-Bt*	2b	2b	2b	0c	0b	10ab	1a	2bc	1a	1b	7ab	3b	12b	5b
Bollgard II*	7b	3b	1b	0c	0b	0b	2a	0c	0a	1b	0c	0b	0c	0c
Bollgard 3*	2b	1b	1b	0c	1b	1b	1a	2bc	1a	3ab	0c	1b	0c	0c
Widestrike 3*	2b	2b	1b	3c	0b	0b	2a	0c	4a	1	1c	1b	0c	0c
Num df	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Den df	21	21	21	21	21	21	21	21	21	21	21	21	21	21
F	3.7	9.3	15.5	18.3	2.3	3.1	1.09	2.67	1.04	1.07	5.1	39.8	21.4	29.1
P	0.0089	<.0001	<.0001	<.0001	.0634	0.0186	0.4063	0.0384	0.4313	0.4138	0.0016	<.0001	<.0001	<.0001

Average percent bloom damage followed by a different letter are significantly different according to a pairwise t-test at an α=0.05
Day 21 in Marianna 2022 was omitted due to zero squares on the plant.
* Sprayed with chlorantraniliprole

Table 13. Mean percent damage of cotton bolls recorded at 4,7,10,14, and 21 days after a chlorantraniliprole application using four cotton cultivars during 2021-2022 growing seasons.

Treatments	Tillar 2021					Tillar 2022					Marianna 2022				
	4	**7**	**10**	**14**	**21**	**4**	**7**	**10**	**14**	**21**	**4**	**7**	**10**	**14**	**21**
Non-Bt	20a	20a	32a	27a	16a	15.2a	2.6ab	14a	15a	25a	12a	23a	24a	28a	17a
Bollgard II	13ab	13ab	27a	19a	7b	4b	6.6a	10ab	12a	5a	3b	0b	1bc	4b	5b
Bollgard 3	3c	1bc	0b	1b	0c	0b	5ab	11ab	25a	26a	0b	0b	0c	0b	0c
WideStrike 3	2c	1bc	2b	0b	1c	0b	4ab	11ab	15a	9a	2b	0b	0c	1b	0c
Non-Bt*	4c	0c	0b	0b	1c	6b	1ab	1b	3a	3a	9a	2b	7b	2b	2bc
Bollgard II*	6bc	2cb	0b	0b	2bc	0b	0b	5ab	0a	3a	1b	0b	0c	1b	1bc
Bollgard 3*	2c	0c	0b	0b	0c	0b	0b	2ab	2a	0a	1b	0b	0c	0b	1bc
Widestrike 3*	3c	1cb	1b	1b	0	1b	3ab	2ab	4a	0a	1b	0b	0c	0b	2bc
Num df	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Den df	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
F	5.2	2.9	9.4	5.8	8.4	3.36	1.24	1.53	1.01	0.99	13.7	14.1	14.7	23.3	13.8
P	0.0014	0.0257	<.0001	0.0007	<.0001	0.0128	0.3298	0.2115	0.4538	0.4638	<.0001	<.0001	<.0001	<.0001	<.0001

Average percent boll damage followed by a different letter are significantly different according to a pairwise t-test at an α=0.05
* Sprayed with chlorantraniliprole

Table 14. Mean percent damage of cotton squares recorded at 4,7,10,14, 21, and 28 days after a foliar insecticide application using three cotton cultivars during 2021-2022 growing seasons.

Treatment	Tillar 2021						Tillar 2022				Marianna 2022	
DAT	4	7	10	14	21	28	4	7	10	14	7	10
Non-Bt	13a	21a	46a	34a	29.5a	53.2a	21a	30a	58a	71a	32a	47a
Chl*	0b	0c	1bc	1c	8bcd	27.7c	13b	18b	27a	12cd	6cd	10c
Chl fb Chl"	4b	2c	1bc	1c	12bc	16.5d	18ab	23ab	30c	26bc	4cd	7cd
Chl fb Ace+Bif"	3b	1c	0c	0c	7cde	19.7dc	14ab	31a	31c	45b	10bc	9c
Ace +Bif*	3b	6b	9b	1c	13b	39.7b	11b	15bc	40c	23c	15b	26b
BGII	3b	2c	3bc	6b	6	10.7def	1c	2d	2d	10cd	3cd	0d
Chl*	1b	1c	1bc	0c	1.5de	5.2efg	0c	1d	1d	0d	0d	0d
Chl fb Chl"	1b	0c	1bc	1c	0g	2.7efg	3c	0d	1d	1d	0d	0d
Chlfb Ace+Bif"	1b	0c	1bc	1c	0.5fg	4efg	0c	0d	2d	1d	0d	0d
Ace +Bif*	0b	0c	3bc	1c	3defg	12.5de	0c	6cd	1d	1d	0d	0d
BG3	1b	0c	0c	0c	0.5fg	0g	1c	0d	1d	0d	0d	0d
Chl*	1b	1c	1c	0c	0g	0.25g	1c	0d	0d	0d	0d	0d
Chl fb Chl"	0b	0c	0c	0c	0g	0g	0c	0d	0d	0d	0d	0d
Chl fb Ace+Bif"	1b	0c	0c	0c	0.5fg	0g	2c	2d	1d	0d	1d	0d
Ace +Bif*	1b	0c	0c	0c	0.5fg	0.5g	1c	0d	0d	1d	1d	0d
Num Df	14	14	14	14	14	14	14	14	14	14	14	14
Den DF	42	42	42	42	42	42	42	42	42	42	42	42
F	3.91	20.7	15.8	42.7	14.4	18.7	8.3	1	13.0	9.3	10.9	20.5
P	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001

Average percent square damage followed by a different letter are significantly different according to a pairwise t-test at an α=0.05
Chl = Chlorantraniliprole (96 g ai/ha) | Ace = Acephate (168 g ai/ha) | Bif = Bifenthrin (148 g ai/ha)
Second application was triggered at 28 DAA in Tillar 2021 and 2022. It was not triggered in Marianna 2022 due to cotton reaching cutout.
Day 14, 21, and 28 were omitted from the 2022 Tillar and Marianna location due to no squares on the plant.
*One insecticide application
" Plots received two insecticide applications

Table 15. Mean percent damage of cotton blooms recorded at 4,7,10,14, 21, and 28 days after a foliar insecticide application using three cotton cultivars during 2021-2022 growing seasons.

Treatment	Tillar 2021						Tillar 2022					Marianna 2022		
DAT	**4**	**7**	**10**	**14**	**21**	**28**	**4**	**7**	**10**	**14**	**21**	**7**	**10**	**14**
Non-Bt	9a	18a	42a	17a	16.5a	22.3a	22a	34a	33a	53a	62.5a	26a	28a	16a
Chl*	3bc	0c	5b	3b	5.5bcd	12.8bc	10cd	13b	16b	17bc	1b	11b	10c	7bc
Chl fb Chl"	2bc	0c	5b	4b	3bcd	9cd	18ab	20b	13bc	13c	1b	4c	8cd	11ab
Chl fb Ace+Bif"	3bc	1c	2b	0b	7b	8.5cde	13bc	13b	6cd	38ab	0b	10b	8cd	5bc
Ace +Bif*	1bc	3bc	8b	2b	15a	17.3ab	9cde	17b	16b	19bc	16b	14b	18b	15a
BGII	5ab	5b	6b	3b	6.5bc	5.2cdef	5defg	0c	4d	6c	8b	2c	2ed	7bc
Chl*	1bc	0c	0b	1b	4bcd	2.7def	0g	0c	0d	1c	0b	0c	0e	0c
Chl fb Chl"	0c	0c	0b	0b	1cd	1.5def	7defg	2c	2d	1c	0b	0c	0e	3c
Chlfb Ace+Bif"	3bc	0c	3b	1b	4bcd	2.2def	1fg	2c	1d	2c	1b	1c	0e	1c
Ace +Bif*	1bc	0c	1b	2b	3bcd	2.5def	2fg	0c	0d	2c	3b	2c	1ed	1c
BG3	0c	0c	0b	0b	1cd	0f	1fg	0c	0d	0c	0b	1c	0e	0c
Chl*	1bc	0c	0b	2b	2bcd	0.25f	3efg	2c	1d	0c	0b	0c	0e	0c
Chl fb Chl"	1bc	0c	1b	0b	0d	0f	0g	1c	1d	0c	0b	0c	0e	0c
Chl fb Ace+Bif"	0c	0c	0b	1b	0.5d	0f	1fg	0c	0d	0c	0b	1c	0e	0c
Ace +Bif*	1bc	0c	0b	0b	0.5d	0.7f	0 g	0c	0d	1c	0b	1c	0e	0c
Num Df	14	14	14	14	14	14	14	14	14	14	14	14	14	14
Den DF	42	42	42	42	42	42	42	42	42	42	42	42	42	42
F	2.5	18.8	13.6	4.4	5.9	6.2	8.2	7.6	10.8	4.7	7.5	15.5	10.6	5.1

P	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01	

Average percent bloom damage followed by a different letter are significantly different according to a pairwise t-test at an α=0.05
Chl = Chlorantraniliprole (96 g ai/ha) | Ace = Acephate (168 g ai/ha) | Bif = Bifenthrin (148 g ai/ha)
Second application was triggered at 28 DAA in Tillar 2021 and 2022. It was not triggered in Marianna 2022 due to cotton reaching cutout.
Day 21, and 28 were omitted from the 2022 Tillar and Marianna location due to no blooms on the plant.
*One insecticide application
“ Plots received two insecticide applications

Table 16. Mean percent damage of cotton bolls recorded at 4,7,10,14, 21, and 28 days after a foliar insecticide application using three cotton cultivars during 2021-2022 growing seasons.

Treatment	Tillar 2021						Tillar 2022						Marianna 2022			
DAT	**4**	**7**	**10**	**14**	**21**	**28**	**4**	**7**	**10**	**14**	**21**	**28**	**7**	**10**	**14**	**21**
Non-Bt	5a	11a	33a	31a	20a	40a	9abcd	30a	32a	50a	62.2a	17a	13a	17a	30a	17a
Chl*	3abc	1bc	1bc	0b	4cdef	25.7b	9abcd	7bcd	13b	9b	2b	3bc	7bc	3b	8bc	2c
Chl fb Chl"	1bc	0c	1bc	1b	7.5bcd	16.2c	17a	9bc	16b	10b	3b	0c	3cde	3b	7c	2c
Chl fb Ace+Bif"	1bc	0c	2bc	0b	4.5cde	13.7c	6bcd	10b	8bcd	39a	2b	1bc	5cd	1b	7c	3c
Ace +Bif*	1bc	3b	10b	0b	11.5b	33a	14ab	12b	11bc	10b	10b	14a	10ab	15a	14b	10b
BGII	3abc	0c	2bc	4b	8bc	12.2cd	4d	3cde	3cd	7b	4b	5b	1de	3b	2cd	12ab
Chl*	4abc	0c	1bc	0b	2ef	5.2de	1d	1de	1d	0b	3b	2bc	0e	2b	2cd	0c
Chl fb Chl"	0c	0c	1bc	0b	1.5ef	3.7e	0d	2de	2d	2b	0b	0c	0e	0b	0d	1c
Chlfb Ace+Bif"	1bc	0c	0c	0b	3.5def	3.2e	2d	2de	1d	5b	0b	0c	0e	1b	2cd	3c
Ace +Bif*	0c	0c	0c	1b	2ef	13.5c	3cd	2de	1d	3b	1b	5b	2de	1b	0d	1c
BG3	1bc	1bc	0c	0b	0f	0e	12abc	0e	0d	1b	0b	0c	0e	0b	0d	0c
Chl*	0c	0c	0c	0b	0f	0e	1d	0e	0d	0b	0b	0c	0e	0b	0d	0c
Chl fb Chl"	0c	0c	0c	0b	0f	0e	4d	2ed	0d	0b	0b	0c	0e	0b	0d	0c
Chl fb Ace+Bif"	0c	0c	0c	0b	1.5ef	0e	1d	0e	0d	0b	0b	0c	0e	0b	0d	0c
Ace +Bif*	0c	0c	0c	0b	0f	0.25e	0d	0e	0d	3b	0b	0c	1de	0b	0d	0c
Num Df	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
Den DF	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42
F	1.7	8.4	6.1	20	13.4	24.9	2.6	11.7	8.8	4.4	7.4	11.9	6.1	7.4	14.8	8.9
P	0.10	<.0001	<.0001	<.0001	<.0001	<.0001	0.0072	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Average percent boll damage followed by a different letter are significantly different according to a pairwise t-test at an α=0.05
Chl = Chlorantraniliprole (96 g ai/ha) | Ace = Acephate (168 g ai/ha) | Bif = Bifenthrin (148 g ai/ha)
Second application was triggered at 28 DAA in Tillar 2021 and 2022. It was not triggered in Marianna 2022 due to cotton reaching cutout.
Day 28 was omitted from the 2022 Marianna location due to cotton reaching cutout.
*One insecticide application
" Plots received two insecticide applications

Chapter III

Evaluation of chlorantraniliprole Detection of cotton *H. zea* using Concentrations of chlorantraniliprole in artificial diet, squares, and bolls

Abstract

Despite widespread use of transgenic *Bacillus thuringiensis* (*Bt*) cotton cultivars, *H. zea* remains a major pest of flowering cotton, and foliar insecticides are often needed to supplement control. Studies were conducted in 2022 at the Lonoke Research and Extension Center in Lonoke, AR to determine if *H. zea* could detect concentrations of chlorantraniliprole using both a diet incorporated and fruiting structure bioassay. Flowering cotton was treated with chlorantraniliprole at a rate of 96 g ai/ha. Squares and bolls were removed from treated plots and untreated plots at 1, 4, and 6 days after treatment. A second instar *H. zea* was placed into a petri dish with either treated, untreated, or a combination of fruiting structures to observed a preference. chlorantraniliprole was incorporated into diet at three rates (812, 3,250, and 13,000 PPB) and were combined with artificial soy-based diet (Southland Products Inc, Lake Village, AR) to yield 1 L of insecticide-treated diet. Incorporated diet was placed in a petri dish and randomized. A cotton pad was submerged in water, folded, and placed in the middle of each petri dish for a source of moisture. One second instar *H. zea* larvae, reared at the Lonoke Research and Extension center, was infested per petri dish. No Choice was observed in either study suggesting *H. zea* cannot detect concentrations of chlorantraniliprole either in diet incorporated assays or when treated fruit is removed from the field. An additional study was conducted in 2022 at the University of Arkansas Lon Mann Cotton Branch Experiment Station near Marianna, AR to determine residual concentrations of foliar applied chlorantraniliprole in cotton blooms, squares, and bolls over time. Slant and lock label tags were placed on the 50 uppermost white blooms,

and were labeled with the date of application. Each week additional uppermost white blooms were tagged after application to ensure significant fruiting structures to sample. Samples were sent to the Mississippi State University Delta Research and Extension Center in Stoneville, Mississippi to determine concentrations of chlorantraniliprole in cotton fruiting structures.

Introduction

Each year *Helicoverpa zea* (Boddie) infests 100% of all cotton planted in Arkansas (Cook, 2021). In 2021, *H. zea* was the second most economically important cotton insect pest in Arkansas behind tarnished plant bug (*Lygus lineolaris*) in terms of yield loss plus cost of control (Cook, 2021). Specific feeding sites on cotton plants are preferred by *H. zea* larvae. Prior to bloom and during the early weeks of blooming, moths often oviposit in the top of the plant canopy. Larvae that hatch from these eggs feed on leaf tissue and small squares near the plant terminal (J. Gore, 2000). As larvae mature, they migrate down the plant, feeding on larger squares and ultimately on bolls (Wilson & Gutierrez, 1980). Larval feeding can reduce yield potential by abscising fruiting structures from the plant and decrease lint quality by feeding on lint within the boll. Bolls developed 15-18 days after flowering will not typically abscise from the plant, however larval feeding allows a passage for moisture and pathogens to enter, causing bolls to develop boll rot (Guinn, 1982, 1986). Regardless, yield losses could still occur from *H. zea* feeding on bolls that do no abscise (J. Gore, 2000).

Prior to the development of transgenic cotton, foliar insecticides were the primary method used to manage *H. zea* in cotton. Synthetic insecticides such as pyrethroids became popular due to their efficacy and cost effectiveness. However, resistance to carbamate, organophosphate, and pyrethroid insecticides has developed in many insects, including *H. zea* (Hopkins, 2010; Pietrantonio et al., 2014). New synthetic compounds such as insect growth

regulators (IGRs), spinosyns, and diamides exhibit novel modes of action to which the insect is still susceptible to. Despite widespread use of transgenic cotton cultivars, supplemental foliar insecticide applications may still be needed to manage some *H. zea* populations.

chlorantraniliprole, a diamide, first registered for use in 2008 has proven to provide premier control for several lepidopteran targets in preliminary field screening trials (Temple, 2009). This class of insecticides is a ryanodine receptor modulator, which activates muscle ryanodine receptors, leading to contraction and paralysis by mediating calcium release into the cytoplasm from intracellular stores (IRAC, 2022). Symptomology includes feeding cessation, lethargy, partial paralysis, and occasionally regurgitation (Cordova et al., 2006).

chlorantraniliprole is primarily absorbed through the stem and transported through the xylem based on observations made in soybean (Adams et al., 2016). chlorantraniliprole, is xylem-mobile through root uptake, which allows the insecticide to move upwards throughout the plant (Lahm et al., 2007). The systemic efficacy of chlorantraniliprole provides great benefits, including effective residual control of caterpillar species in crops such as brassicas, soybeans, and cotton (Smith, 2022). Diamides, including chlorantraniliprole have low activity on non-target beneficial insects and reduced mammalian toxicity (Lahm et al., 2007). chlorantraniliprole demonstrated very good activity at relatively low rates against all three major caterpillar pests, including *H. zea*, *H. zea*; tobacco budworm, *Heliothis virescens* (F.), and fall armyworm, *Spodoptera frugiperda* (Smith) (Temple, 2009).

Gore et al. (2002) conducted an experiment using a non-*Bt* and Bollgard (Monsanto Co., St. Louis, MO) cotton cultivar containing the cry1Ac gene to determine if *H. zea* larvae could detect and avoid *B. thuringiensis* proteins. Gore et al. (2002) found that *H. zea* larvae began migrating away from Bollgard cotton terminals within 1 h of being infested and within 6 h <

10% of larvae remained in Bollgard terminals. *H. zea* larvae were found to exhibit a dispersal pattern on cotton containing *B. thuringiensis* whereas little to no dispersal occurred on non-*Bt* cotton (Gore et al., 2002). The objective of this study was to determine if *H. zea* can detect chlorantraniliprole concentrations in a laboratory and field setting.

Materials and Methods

Insect Rearing

Laboratory colonies of *H. zea* used in the choice bioassays were maintained at the Lonoke Extension and Research facility. The colony originated from Mississippi State University, collected from non-*Bt* corn ears in 2006, and wild individuals were added to the colony on a biannual basis to maintain genetic diversity (Ngomane et al., 2022). The colony was reared under recommended conditions of 25°C, 80% relative humidity, and 16:8 (L:D) photoperiod. At pupation, approximately 40 pupae were placed into a 5.7 L plastic storage box and covered with cheesecloth, which acted as a removeable oviposition substrate location for moths. Egg sheets were collected and placed into 3.8 L self-sealing bags (Ziploc®, S.C. Johnson & Son, Inc., Racine, WI). Upon hatching, approximately 100 larvae were transferred into 473 mL plastic deli containers (Fabr-Kai Corp, Kalamazoo, MI) filled with artificial soy-based diet (Southland Products Inc, Lake Village, AR) and covered. Transferred larvae were returned to the rearing room until larvae reached second instar at which point assays were initiated.

Fruiting Structure Application

A choice assay was conducted in 2022 in Lonoke, AR at the Lonoke Research and Extension Center to determine if *H. zea* could detect concentrations of chlorantraniliprole in cotton squares and bolls from infield foliar applications. Field plots were established at the

University of Arkansas Lon Mann Cotton Branch Experiment Station near Marianna, AR. Plots were planted using a non-*Bt* (DP 1822 XF, Bayer CropScience, St. Louis, Mo). All trials were planted in a randomized complete block design with 4 replications with a John Deere Max Emerge 2 7300, four row planter modified with Almaco 31 cell cones for plot planting. Each variety was planted at 135,850 seed ha^{-1} into conventionally tilled raised beds between 16 May and 18 May. Plot size was four rows, 96.5 cm apart and 12.2 m long. Two plots were left untreated, while two plots with the same plot size were treated with 96 g ai/ha of chlorantraniliprole (Prevathon 0.43 SC, FMC Corporation; Philadelphia, PA). chlorantraniliprole was applied on 4 Aug using a Mudmaster high clearance sprayer fitted with TXVS-6 hollow cone nozzles at 50 cm spacing with a spray volume of 94 L/ha, at 276 kpa. At 1,4, and 6 days after treatment (DAT), 90 untreated fruiting structures and 90 treated fruiting structures, were pulled from the upper one-third of plants in the center two rows. Gloves were changed between plots to reduce the risk of cross contamination. Samples were place in 946 mL self-sealed plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, WI) and transported back to the laboratory in a cooler with ice.

Three choices were used in this experiment. The first treatment consisted of two untreated fruiting structures placed in a petri dish. The second treatment was one untreated and one chlorantraniliprole treated fruiting structure, while the third treatment used two chlorantraniliprole treated fruiting structures. This experiment was conducted three times, with each treatment being replicated 30 times for a total of 90 samples per run (n=90), and a pooled sample size of 270 observations (n=270).

In the lab bracts were removed from each fruiting structure. Gloves were used to extract fruiting structure from the self-sealed bags. Two fruiting structures were placed into individual

self-sealing 50 x 9mm petri dishes (VWR, Avantor, Inc. Radnor, PA). Fruiting structures were placed on opposite edges of each dish. A cotton pad (Swisspers, Parkdale, Inc. Gastonia, NC) was submerged in water, folded, and placed in the middle of each petri dish for a source of moisture. A second instar larvae was placed in the center of each cotton pad with a fine bristle artist's paint brush to minimize a preference based on proximity to a specific fruiting structure. If larvae were not feeding upon a fruiting structure, they were recorded as having made "no choice".

Diet Incorporated Bioassays

Diet incorporated concentration-choice bioassays were conducted in 2022 at the Lonoke Extension Center in Lonoke, Arkansas to determine if lab colony corn earworms could detect concentrations of a commercial formulation of chlorantraniliprole (Prevathon®, FMC Corporation, Philadelphia, PA). Dilutions were prepared by adding 1 ml of chlorantraniliprole to 1 liter of water. Three rates of the stock solution, 1.36 ml, 5.425 ml, and 21.7 ml were combined with Stonefly *Heliothis* diet (Southland Products Inc, Lake Village, AR) to yield 1 L of insecticide-treated diet concentrations of 812 ppb, 3,250 ppb, and 13,000 ppb of chlorantraniliprole, respectively. Diet was blended and allowed to cool for 3-4 minutes before chlorantraniliprole solution was incorporated and blended for an additional 30 seconds to allow insecticide to disperse evenly. Gloves were worn and changed between each concentration to reduce the risk of cross contamination. Treated diet was placed into a 53.3 cm x 33.0 cm disposable aluminum pan (GLAD, The Clorox Company, Oakland, CA) and allowed to cool. Diet was then cut with a 1.9 cm hole punch tool by concentration using gloves and washing hole punch tool between concentrations to avoid cross contamination between treatments. Diet plugs measuring 1.3 cm tall were placed in square individual self-sealing 90 x 15mm petri dishes

(VWR, Avantor, Inc. Radnor, PA) and randomized. A cotton pad (Swisspers, Parkdale, Inc. Gastonia, NC) was submerged in water, folded, and placed in the middle of each petri dish for a source of moisture. One second instar corn earworm larvae was infested per petri dish. Larvae was placed in the center of each cotton pad with a fine bristle paint brush to minimize a preference based on proximity to an individual treated diet. Larvae were evaluated 48 hours after infestation for choice. Larvae that were not feeding upon a cutout of diet were recorded as having made "no choice". Each concentration was replicated 30 times. Each run had a total of 90 samples per run (n=90). This experiment was run three times, making total sample size of pooled runs n=270.

chlorantraniliprole Concentrations in Cotton Fruiting Structures

A study was conducted in 2022 at the University of Arkansas Lon Mann Cotton Branch Experiment Station near Marianna, AR to determine residual concentrations of foliar applied chlorantraniliprole in cotton blooms, squares, and bolls over time. All field components and chlorantraniliprole applications were similar to the fruiting structure assay. chlorantraniliprole application was initiated first week of bloom. Preceding the foliar application, 50 uppermost white blooms, were labeled with the date of application using slant and lock label tags (A.M. Leonard, Piqua, OH). Approximately 40 additional white blooms in the upper one-third of the plant were tagged each week after application to ensure sufficient fruiting structures for data collection. Labeling and tagging were conducted to determine residual concentrations and dispersal of chlorantraniliprole by the plant using days after treatment (DAT) in previous undeveloped fruiting structures.

At 3, 7, 14, 21, 27 days after treatment (DAT), ten squares, blooms, and bolls with the appropriate dates were removed from each plot. Gloves were worn and changed between plots to

reduce the risk of cross contamination. Samples were place in 946 mL self-sealed plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, WI) and transported back to the laboratory. Bracts were removed from each fruiting structure prior to being placed in a freezer set to -18° C. Samples were sent to the Mississippi State University Delta Research and Extension Center in Stoneville, Mississippi to determine concentrations of chlorantraniliprole in cotton fruiting structures.

Chemical Analysis

Cotton flower, square, and boll samples were analyzed using a modified QuEChERS by LC/MS/MS and GC/MS/MS procedure developed by Anastassiades and Lehotay (2003), and results were displayed in parts per billion (PPB) of active ingredient. Flower, square, and boll samples were ground into a powder and 5g of the sample was deposited into a 50mL polypropylene tube. Additionally, 5g of clean, lab grown samples were placed into two 50mL polypropylene tubes for a “blank” and “spike” sample. Spike samples were given adequate concentrations of insecticides to be tested to ensure concise readings and the blank sample were left untreated. Ceramic beads were placed in each tube for homogenizing the samples when centrifuging. Additionally, 10mL of high-performance liquid chromatography water were deposited in the tubes. A GenoGrind (SPEX Sample Prep, Metuchen, NJ) plant tissue homogenizer was used to centrifuge all samples at 1000 RPM for five minutes. Following the first round of centrifuging, each sample received 10mL of acetonitrile (ACN), which allows extraction of the active ingredient, and were centrifuged again for five minutes. MgSO4 (anhydrous magnesium sulfate) was then added to samples to separate the active ingredient from plant material. Additional five minutes of centrifuging was needed to separated water and ACN. Samples were placed back into the 50 GenoGrind and centrifuging time and RPM was increased

to ten minutes and 4000, respectively. Following this final round of centrifuging, complete separation of the mixture was achieved with the top layer of liquid containing the residual active ingredient. 1mL of the extracted liquid was placed into 15mL polypropylene tubes. Tubes containing the extracted liquid were placed into an auto sampler vial with a PTFE/PVDF filter and analyzed using a LC/MS/MS or GC/MS/MS for GC-amenable pesticides. Recovery of residual insecticide ranged between 85-101% (mostly >95%) (Anastassiades et al., 2003).

Results

Square and Boll Application

No choice was made among the choices presented suggesting that *H. zea* cannot detect concentrations of chlorantraniliprole in squares or bolls after a foliar application. For squares, the no choice treatment had the lowest response numerically, which indicates the larvae did not choose a square or were not feeding upon one at the time of rating (Table 17). There was a higher numerical percentage of *H. zea* choosing squares and bolls placed on the left side of the petri dish in the untreated treatment when compared to all other treatments (Table 17-18). A positive numerical response was observed for treated bolls when compared to all other treatments except for fruit that was untreated and placed on the left hand side of the petri dish (Table 17-18). Untreated bolls placed on the right-hand side of the petri dish had the lowest numerical percentage response compared to all other treatments (Table 18). Preliminary data suggest chlorantraniliprole is not detected by *H. zea* larvae, but further evaluations are needed to confirm these findings.

Diet Incorporated Bioassay

Between the five different choices the larvae were presented with, there were no significant differences across all treatments (F=1.77, *P*=0.147)(Table 19). These data suggest that *H. zea* cannot detect chlorantraniliprole.

Cotton Fruiting Structure Study

There was a significant interaction between age of fruiting structure and DAT (F=6.91, df=14,40, p<0.01) for concentrations of chlorantraniliprole detected. At 3 DAT, the highest detection of active ingredient, 617 PPB, were found in squares (Table 20). At 3 DAT, the second highest concentration level, 226 PPB, was detected in Boll1 which is a 3-day old boll. Generally, concentrations decreased over time as the age of the fruiting structure increased. However, an increase in concentration was observed in the blooms from 3 DAT (99.86 PPB) to 7 DAT (229 PPB) (Table 20). Concentrations detected at 3, 7, 14, and 21 DAT decreased over time except in blooms from 3 DAT to 7 DAT. Concentrations of chlorantraniliprole were detected in blooms at 27 DAT in small amounts, however not detection occurred in squares and bolls (Table 20).

Discussion

Typically, *H. zea* feeding is of most concern to marketable structures of cotton such as squares and bolls. Leaves of cotton plants are the most attractive for *H. zea* oviposition, and many eggs are found on leaves deep in the canopy (Lewis R Braswell et al., 2019). Once hatched on leaves, first instar *H. zea* larvae feed on fruiting structures near the oviposition site (Lewis R Braswell et al., 2019). In a study by Smith et al. (2022), chlorantraniliprole shows potential to cause residual mortality up to 28 DAT of *H. zea* migrating from leaves to fruiting structures.

Results from Adams et al. (2016), indicates chlorantraniliprole moved systemically to vegetation in soybean and provided some control of *H. zea*, but no mortality was recorded in

reproductive structures. Results from the cotton fruiting structure chemical analysis study indicate no concentrations of chlorantraniliprole in the fruiting structures past 14 DAT would cause mortality of *H. zea.* (Adams et al., 2016; Lewis R. Braswell et al., 2019; Lewis R Braswell et al., 2019; Smith, 2022). Generally, as sampling date increased and rate decreased, concentrations of chlorantraniliprole decreased. Concentrations at 3 DAT were high compared to other sampling dates. This is most likely due to spray coverage and age of fruit. In this study, concentrations decreased over time as the compound degraded and diluted through the plant. Structures on the plant at the time of application had higher concentrations of chlorantraniliprole throughout the four weeks compared to structures the plant produced during the four-week time span. Based on these concentration levels, all fruiting structures will become more susceptible over time, but flowers will be the most susceptible. Smith et al. (2022), detected concentrations of chlorantraniliprole in cotton flower petals but not in anthers. Some *H. zea* control might be expected up to 14 DAT and *H. zea* mortality up to 47% might be expected in cotton flowers undeveloped at the time of a chlorantraniliprole application (Smith, 2022). On the basis of this observation, *H. zea* feeding is almost always exclusive to the cotton anthers and not the petals themselves because survival rates are generally greater on anthers than petals (J Gore, 2000; Smith, 2022).

Results similar to those found in the current study have been observed previously. Smith et al. (2022) conducted an experiment using different rates of chlorantraniliprole applied to plots at the second week of bloom. Smith et al. (2022) found chlorantraniliprole concentrations persisted through all sampling dates and nearly all concentrations of chlorantraniliprole detected were in greater concentrations than what was used in a diet incorporated assay, where mortality occurred.

Most heliothines oviposit in the top third of the canopy on both non-*Bt* and *Bt* cotton; however, oviposition location has historically not influenced the ability of larvae to move to and establish on the reproductive tissues (squares and bolls) that are their preferred feeding structures (Wilson & Gutierrez, 1980; Farrar Jr & Bradley Jr, 1985). Contrary to adult oviposition behavior, *H. zea* larval behavior can be influenced by the presence of *Bt* toxins (Lewis R Braswell et al., 2019). Resistant insects may detect or recognize a danger and avoid the toxin. This mechanism of resistance is known as behavioral resistance, the insect populations may develop the ability to avoid or reduce lethal insecticide exposure (Dang et al., 2017). It has been reported for several classes of insecticides, including organochlorines, organophosphates, carbamates and pyrethroids (Dang et al., 2017). However, *H. zea* larvae do not have the ability to detect concentrations of chlorantraniliprole. Residual concentrations of chlorantraniliprole were detected throughout multiple fruiting structures in this study, and this compound shows potential to have residual up to 28 DAT. Results from the cotton fruiting structure and diet choice assay study is insignificant and shows no indication that *H. zea* can detect concentrations of chlorantraniliprole applied by a foliar application. Unlike *Bt* toxins, *H. zea* cannot detect chlorantraniliprole and should continue to serve as a supplemental control agent. *Bt* cotton cultivars are the primary management option for *H. zea* and insecticides, mainly diamides, should be used as a supplemental control agent (Reisig & Kurtz, 2018; Crow et al., 2021).

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Table 17. Mean percent of *H. zea* choice assays conducted on chlorantraniliprole treated cotton squares conducted in Lonoke, AR 2022

Treatment	Mean
Untreated	39 % a
Treated	40 % a
No Choice	20 % b
NumDf	2
DenDf	24
F	7.91
P	0.0023
Average percent number of *H. zea* followed by a different letter are significantly different according to a pairwise t-test at an α=0.05	

Table 18. Mean percent of *H. zea* choice assays conducted on chlorantraniliprole treated cotton bolls conducted in Lonoke, AR 2022

Treatment†	Mean
Untreated	38 % a
Treated	39 % a
No Choice	23 % b
NumDf	2
DenDf	24
F	7.69
P	0.0026

Average percent number of *H. zea* followed by a different letter are significantly different according to a pairwise t-test at an α=0.05

Table 19. Percent of *H. zea* larvae and their choice of artificial diet treated with concentrations of chlorantraniliprole conducted in Lonoke, AR 2022

Rate	Mean
0	17 %
812 PPB	23 %
3,250 PPB	14 %
13,000 PPB	18 %
No Choice	25 %
NumDf	4
DenDf	55
F	1.77
P	0.1471

Table 20. Mean chlorantraniliprole concentrations in parts per billion from in-field application for multiple cotton fruiting structures multiple days after application for studies conducted in Marianna, AR in 2022.

Fruit	Days After Treatment				
	3	**7**	**14**	**21**	**27**
Bloom	99.9	229.0	30.5	12.7	5.3
Square	617.0	60.6	3.7	3.6	N/A
Boll 1† (7/19)	226.0	37.2	1.5	1.3	N/A
Boll 2† (7/22)	N/A	39.9	N/A	1.6	N/A
Boll 3† (7/26)	N/A	N/A	6.0	3.9	N/A
Boll 4† (8/3)	N/A	N/A	N/A	3.4	N/A

† Days After Treatment represents each day fruiting structures were collected.
†Boll 1 was tagged on 7/19 as an uppermost white bloom
† Boll 2 was tagged on 7/22 as an uppermost white bloom
† Boll 3 was tagged on 7/26 as an uppermost white bloom
† Boll 4 was tagged on 8/3 as an uppermost white bloom
Boll 5 was removed due to no concentration readings

Conclusion

Helicoverpa zea infestation pressure was low in 2022 compared to previous years. Seasonal fruit damage was relatively low at the Tillar and Marianna locations. Based on findings from this experiment, dual gene appears to provide adequate control of *H. zea* under low pressure. However, dual gene alone may not provide satisfactory control of *H. zea* when pressure is moderate or greater and supplemental foliar insecticide applications may be required. Three gene cultivars appear to provide sufficient control of *H. zea* but should still be monitored to prevent yield loss. Growers applying chlorantraniliprole will achieve adequate control in two-gene cultivars. When planting a three gene cultivar, growers are breaking even >70% of the time with an average profit of $400 per hectare.

Helicoverpa zea larvae can be influenced by the presence of *Bt* toxins and avoid the toxin. This mechanism of resistance is known as behavioral resistance, the insect populations may develop the ability to avoid or reduce lethal insecticide exposure. Results from the cotton fruiting structure and diet choice assay study is insignificant and shows no indication that *H. zea* can detect concentrations of chlorantraniliprole applied by a foliar application. Unlike *Bt* toxins, *H. zea* cannot detect chlorantraniliprole and should continue to serve as a supplemental control agent.

In conclusion, due to recently documented resistance in *H. zea* to multiple cry proteins, growers should budget at least one diamide insecticide application when planting anything other than a three gene variety to prevent yield loss. Three gene provides exceptional control and decreases the amount of supplemental foliar applications required for *H. zea*. Chlorantraniliprole cannot be detected by *H. zea* and should continue to be used as a control agent for *H. zea*.