ARTHROPOD MANAGEMENT & APPLIED ECOLOGY

Efficacy of Bt Toxins and Foliar Insecticides Against Bollworm, *Helicoverpa zea* (Boddie), in Dried Flower Corollas of Cotton

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ABSTRACT

Foliar insecticides and insecticidal protein from Bacillus thuringiensis (Bt) in transgenic cotton are common tools used for bollworm management in cotton. Efficacy of Bt proteins and foliar insecticides can be dependent upon larval location in the plant canopy and time of year. Floral structures are known to be a common food source for bollworm. Floral components can complicate bollworm control with foliar insecticides and transgenic cotton by protecting larvae from contact with formulated insecticides and lower concentrations of Bt proteins. Mortality was measured to evaluate the effects of Bt expression and foliar insecticides in flowers of non-Bt, two-gene Bt, and three-gene Bt cotton varieties. Expression of Bt protein in white flowers provided some efficacy after three days. Bollworm mortality at three days after infestation was less than 50% for all varieties in an unsprayed environment. Larval mortality on Bollgard 3 flowers was higher than that on Bollgard II flowers. Surviving larvae feeding on Bollgard 3 flowers weighed less than larvae that fed on Bollgard II flowers, and larvae that fed on Bollgard II flowers weighed less than those that fed on non-Bt flowers. The use of chlorantraniliprole or methoxyfenozide + spinetoram provided some control of bollworms three days after application to wilting flower corollas (bloom tags). Mortality ranged from 41.9% following application of chlorantraniliprole to 61.0% following application of methoxyfenozide + spinetoram. Results from this study will be used to improve integrated pest management programs for bollworm management in cotton.

B ollworm, *Helicoverpa zea* (Boddie), is an economically important insect pest of cotton in most regions of the U.S. Bollworm larvae feed on flower buds (squares), fresh flowers, wilting flower corollas (bloom tags), and small bolls (Farrar and Bradley, 1985). Feeding by bollworm can cause abscission of fruiting forms, delayed maturity, and reduced yield (Gore et al., 2008). Growers commonly manage bollworm in cotton by planting transgenic varieties that express various insecticidal proteins and applying foliar insecticides when local action thresholds are met.

Foliar insecticides were used extensively for bollworm control prior to the introduction of transgenic cotton varieties that express insecticidal proteins derived from *Bacillus thuringiensis* (Bt) var. *kurstaki*, a soil-dwelling bacterium (Reisig et al., 2018). Bollworm and tobacco budworm, *Chloridea virescens* (F.), were the main lepidopteran pests targeted with foliar insecticides in cotton (Clower, 1980; Staetz, 1985). Though foliar insecticides provided control of bollworm, negative effects on nontarget organisms (Baker, 1982; Wu et al., 2018) and poisoning of applicators (Calvert et al., 2008) were observed, along with development of resistance in many insects (Baker, 1982).

Insecticide resistance to chlorinated hydrocarbons in tobacco budworm and bollworm populations was detected in the early 1950s (Graves et al., 1963, 1967; Ivy and Scales, 1954; Sparks, 1981) and to organophosphates in the 1960s (Plapp, 1971). Pyrethroids were used frequently for the control of tobacco budworm and bollworm through the 1970s and 1980s (Snodgrass and Scott, 2000). Due to the frequent use of pyrethroids, decline in efficacy against tobacco budworm and bollworm also was

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observed because of resistance development (Abd-Elghafar et al., 1993; Jacobson et al., 2009; Pietrantonio et al., 2007; Stadelbacher et al., 1990).

Transgenic Bt cotton was introduced in 1996 and provided a novel form of control and method to combat insecticide resistance in tobacco budworm and bollworm. An overall decrease in insecticide use was observed as Bt cotton acreage increased (Reisig et al., 2018), primarily due to excellent control of tobacco budworm with plant-incorporated proteins (Naranjo, 2011). Plants expressing Bt proteins provided environmental and economic benefits by decreasing the number of insecticide applications (Betz et al., 2000; Smith, 1997; Wilkins et al., 2000), which reduced harmful effects on non-target organisms (Betz et al., 2000) and improved yield (Gianessi and Carpenter, 1999). Bt cotton quickly became a primary control method for bollworm and other lepidopteran pests. However, limitations of Bt technology were observed shortly after introduction. Upon commercial introduction of Bt cotton expressing Cry1Ac, bollworm larvae behaved differently than they did on non-Bt cotton (Gore et al., 2002). Larvae were observed surviving lower in the plant canopy of Bt cotton and were commonly observed surviving in flowers and bloom tags. Behavior of adults was likely a contributing factor in overall establishment of larvae lower in the canopy, as they were commonly found laying eggs on plant structures lower in the canopy (Braswell et al., 2019) and on the various fruiting structures, including flowers. Lower Bt concentrations were observed in flowers in relation to the rest of the plant, allowing higher levels of bollworm survival (Gore et al., 2001). Development of cotton varieties that incorporated multiple Bt proteins, such as Cry2Ab with Cry1Ac, provided more consistent bollworm control, but higher survival on floral components was observed when compared with other fruiting forms (Gore et al., 2008).

Bollworm damage to two-gene cotton has become more severe in recent years, likely due to the development of resistance to Bt proteins in some populations (Fleming et al., 2018; Godbold et al., 2022; Reisig et al., 2018). Larval survival in flowers of cotton expressing two Bt proteins has likely increased as a result. The development of Bt resistance and survival of bollworm populations has increased the need for supplemental control with foliar insecticides (Reisig et al., 2018). Currently, multiple modes of action, as described by the Insecticide Resistance Action Committee, are used in rotational strategies to prevent or delay resistance development to insecticides (https://irac-online.org/modes-of-action/, verified 27 Oct. 2022). Common insecticides currently used include chlorantraniliprole from the diamide class (Prevathon 0.43 SC; FMC Corporation, Philadelphia, PA), and a premix of methoxyfenozide and spinetoram from the diacylhydrazine and spinosyn classes, respectively (Intrepid EdgeTM; CortevaTM AgriScience, Indianapolis, IN).

Beginning in 2018, cotton cultivars expressing a third insecticidal protein, Vip3A, became available. The addition of Vip3A provided a novel mode of action (Lee et al., 2003) to aid in bollworm control. The Vip3A protein binds to a different target site within the midgut of bollworm than Cry proteins and currently provides better control than two-gene cultivars (Gupta et al., 2021). However, based on development of resistance to previously used foliar insecticides and transgenic crops, additional forms of control are likely needed to help delay resistance development to Vip3A and current foliar insecticides.

Due to larval movement (Gore et al., 2002) and survival in flowers (Gore et al., 2001), larvae are likely to be found in flowers or bloom tags at some point during development. Previous research investigating bollworm survival in white flowers was conducted prior to the development of resistance, and the current extent of larval survival in Bt cotton flowers is unknown. The efficacy of foliar insecticides on larvae residing in bloom tags is also not known. Flowers remain open for a few days before closing and becoming a dried bloom tag on the outer surface of the developing boll (Mauney, 2012). Larvae feeding in flowers during this process can be concealed inside the bloom tag until they feed into the boll or out of the flowers themselves. Once sealed, the bloom tag can serve as a protective structure for bollworm larvae. To improve our understanding of bollworm use of and survival in cotton blooms and bloom tags, experiments were conducted during 2018 and 2019 at the Mississippi State University Delta Research and Extension Center in Stoneville, MS, to evaluate the effects of bollworm feeding on Bollgard II (Cry1Ac, Cry2Ab) and Bollgard 3 (Cry1Ac, Cry2Ab, VIP3A) cotton flowers on larval mortality and development in comparison with larval response to feeding on non-Bt flowers. Additionally, efficacy of foliar insecticides against bollworm inside bloom tags at the time of insecticide application was evaluated.

MATERIALS AND METHODS

Bollworm Collections and Rearing. An early season collection of bollworm larvae was made using a sweep net from crimson clover, Trifolium incarnatum L., during early May 2018 and 2019. An additional collection was made by hand during late June to early July of each year from milk-stage non-Bt (Dekalb DKC 67-70; Bayer CropScience, St. Louis, MO) and Bt (Dekalb DKC 67-72 VT2P) field corn, Zea mays L. Bt proteins expressed in the Bt corn hybrid were Cry1A.105 and Cry2Ab2. For collections, late instar larvae were placed individually in 59.2-mL cups (Solo®; Dart Container Corp., Mason, MI) containing Stonefly Heliothis diet (Ward's Science, Rochester, NY). Larvae were placed in a controlled climate environment for the establishment of a colony. The temperature was set to 26.7 °C at 80% relative humidity and a light:dark ratio of 16:8 h. Larvae were allowed to feed on diet until reaching the pupal stage. Once larvae pupated, the pupae were removed from the cups, rinsed with a 5% solution of sodium hypochlorite and water, and placed into 3.79-L cardboard buckets. The top of each bucket was covered with cheesecloth that was held in place by the lid with the center portion removed. Cheesecloth provided a removable and replaceable surface for bollworm adults to oviposit. Cheesecloth was removed daily and placed in 3.79-L self-sealing plastic bags (Ziploc[®]; S.C. Johnson & Son, Inc., Racine, WI) labeled according to generation and the origin of the colony. Upon hatching, neonates were transferred with a small, fine-bristled paintbrush into 59.2-mL cups containing Stonefly Heliothis diet.

Bt Floral Tissue Bioassay. Deltapine cotton varieties were planted at the Delta Research and Extension Center in Stoneville, MS, during 2018 and 2019. Three different cultivars were used for the experiment and represented non-Bt (Deltapine DP1822 XF; Bayer CropScience, St. Louis, MO), Bollgard II (Deltapine DP1646 B2XF), and Bollgard 3 (Deltapine DP1835 B3XF). White flowers were removed during the late flowering stage (≤ 5 nodes above white flower) of the different varieties by cutting each flower with scissors at the base of the pedicel next to the main stem so that the flowers could be placed into water picks.

Water picks were created using 15-mL centrifuge tubes (Globe Scientific Inc., Mahwah, NJ) filled with tap water. Rubber caps, with a small diameter opening were placed over the open end of the centrifuge tube. Tubes were inserted into the lids of 355-mL clear plastic cups (Zeml[©], Albany, NY) to serve as a base with the top of the lid facing downward. Water picks (centrifuge tube and cap) were then placed into wooden test tube holders (38.1 x 1.91cm). Flowers (attached to stems) collected from the field were placed into water picks by inserting the stems into the small opening of the rubber caps. Tube and cap combinations provided support and moisture to flowers during the experiment. Bollworms originating from non-Bt and Bt corn hybrids were used to infest these flowers. One first-instar bollworm larva was placed into each flower. The 355-mL cups were then placed over each flower and secured to the lid. Holes were punched in the base of cups to allow the escape of moisture and to promote the drying of flowers. Larvae were allowed to remain in the flower for 3 d at which time mortality rate and weight of survivors were recorded. Weights of surviving larvae were measured on an AL54 analytical balance (Mettler-Toledo, Columbus, OH). Larvae were then placed individually in 59.2mL cups containing Stonefly Heliothis diet. After 8 d, weights of surviving larvae were recorded to determine weight gain after 3 d of exposure to cotton blooms followed by feeding on artificial diet.

Analyses. The experiment was replicated eight times over 2 yr. Each replicate was conducted on a separate date and consisted of 10 larvae from the second laboratory generation of non-Bt and Bt corn colonies. All replications from 2018 contained larvae from each colony. During 2019, only one replication contained larvae from both colonies due to rearing problems with the Bt corn colony, so the last three replications during 2019 used only bollworm larvae originating from non-Bt corn. All data were analyzed with a generalized linear mixed model analysis of variance (PROC GLIMMIX, SAS ver. 9.4; SAS Institute, Cary, NC) with a Gaussian distribution and identity link function. Degrees of freedom were calculated using the Kenward-Roger method (Kenward and Roger, 1997). Percentage larval mortality was transformed (log10 + 1) to normalize the data prior to analysis (Zar, 1999). Insect colony, Bt technology (non-Bt, Bollgard II, or Bollgard 3), and the interaction were considered fixed effects in the model. Replication, replication nested in year, and colony nested in year were considered random effects in the model. Means and standard errors were calculated with a PROC MEANS statement. LSMEANS were separated according to the Tukey's HSD at $\alpha = 0.05$ (Tukey, 1953).

Insecticide Bioassay. White flowers from a non-Bt cotton variety (DeltaPine DP1822 XF) were removed from field-grown plants with scissors ensuring that the stem remained intact with the rest of the fruiting structure. Flowers were placed in water picks as described above. Once in the water picks, one first-instar bollworm larva was placed into each flower. Each replication contained 10 individual larvae and flowers for each of four treatments. One day $(24 \pm 2 h)$ after larvae were placed in flowers, insecticide applications were made using a CO₂pressured backpack sprayer with four XR11001VS spray nozzles (TeeJet® Technologies, Glendale Heights, IL) in a volume of 94 L ha⁻¹ and 206 kPa. Insecticide applications were made 24 h after infestation to allow the flower to naturally close around the larva and provide protection from direct spray. Two rates of chlorantraniliprole (Prevathon) were used during the experiment (0.053 kg ai ha⁻¹ and 0.075 kg ai ha⁻¹) and one rate of a premix of methoxyfenozide plus spinetoram (Intrepid Edge) that resulted in 0.175 kg ai ha⁻¹ and 0.035 kg ai ha⁻¹ for each of those active ingredients, respectively. A non-treated control was included that was not sprayed with any insecticide. Clean water was sprayed through the handheld sprayer between each treatment application to remove insecticide residue. Larval mortality was assessed 3 d after the insecticide application. Bloom tags (dry flowers) and bolls underneath bloom tags were dissected and visually inspected under a magnifying glass to identify larvae and to determine levels of mortality.

Analyses. The experiment was replicated seven times over the 2-yr period with each replication containing 10 flowers and larvae. Each replication was conducted on separate days with freshly mixed insecticides. All data were analyzed with a generalized linear mixed model analysis of variance (PROC GLIM-MIX). Percentage larval mortality was transformed $(\log 10 + 1)$ to normalize the data prior to analysis (Zar, 1999). Degrees of freedom were calculated using the Kenward-Roger method (Kenward and Roger, 1997). Insecticide was considered the fixed effect in the model. Replication and replication nested in year were considered random in the model. Means and standard errors were calculated with a PROC MEANS statement. LSMEANS were separated according to the Tukey's HSD at $\alpha = 0.05$ (Tukey, 1953).

RESULTS

Bt Floral Tissue Bioassay. No differences in bollworm mortality were observed between colonies (F = 0.87; df = 1, 2.1; p = 0.45), and there was no interaction between colony and Bt technology (F = 0.60; df = 2, 25.5; p = 0.56). Bt technologies caused bollworm mortality after the 3-d feeding period (F = 50.37; df = 2, 25.5; p <0.01). Despite the occurrence of resistance to Cry proteins broadly observed across multiple crops (Dively et al., 2016; Reisig et al., 2018), greater bollworm mortality was observed on Bollgard II and Bollgard 3 flowers than on non-Bt cotton flowers (Fig. 1). Greater bollworm mortality was observed on Bollgard 3 cotton compared with that on Bollgard II cotton (Fig. 1).



Figure 1. Bollworm mortality on non-Bt, Bollgard II, and Bollgard 3 cotton flowers. Percent mortality \pm SEM 3 d after infestation. Stoneville, MS, 2018 and 2019. Means separation was conducted with transformed (log10) data. Means and standard errors (%) presented are not transformed. Columns containing the same letter are not significantly different (Tukey-Kramer HSD test, $\alpha = 0.05$).

Insect colony origin (F = 0.60; d.f. = 1, 1; p = 0.58) did not affect larval weights after 3 d, and there was no colony-by-Bt-technology interaction (F = 2.14; d.f. = 2, 25.3; p = 0.14) for larval weights. Bt technology (F = 73.05; d.f. = 2, 25.3; p < 0.01) affected larval weights 3 d after infestation. Larvae that survived on flowers from varieties containing Bt proteins weighed less than larvae that fed on flowers from non-Bt cotton (Fig. 2). Larval weight also differed among Bt varieties. Surviving bollworm larvae that fed on flowers from Bollgard 3 cotton weighed less than surviving bollworm larvae that fed on flowers from Bollgard 3 cotton weighed less than surviving bollworm larvae that fed on flowers from Bollgard II cotton after 3 d.

Larval weight gain (Fig. 3) after 8 d on *Heliothis* diet was affected by Bt technology (F = 5.06; d.f. =

2, 25.3; p = 0.01) but not by insect colony origin (F = 0.01; d.f. = 1, 1.73; p = 0.93) or the colony-bytreatment interaction (F = 0.89; d.f. = 2, 25.26; p = 0.42). No differences in larval weight gains were observed between bollworm larvae feeding on Bt varieties, but larval weight gain from non-Bt cotton was greater than from Bollgard 3.



Figure 2. Bollworm larval weights after feeding in non-Bt, Bollgard II, and Bollgard 3 cotton flowers for 3 d. Weight of surviving larvae in milligrams \pm SEM per larva 3 d after infestation. Stoneville, MS, 2018 and 2019. Columns containing the same letter are not significantly different (Tukey-Kramer HSD test, $\alpha = 0.05$).



Figure 3. Bollworm larval weight gain from feeding on *Heliothis* diet for 8 d after removal from 3-d exposure to non-Bt, Bollgard II, and Bollgard 3 cotton flowers. Weight gain per surviving larva in milligrams \pm SEM 11 d after infestation. Stoneville, MS, 2018 and 2019. Columns containing the same letter are not significantly different (Tukey-Kramer HSD test, $\alpha = 0.05$).

Insecticide Bioassay. All insecticide treatments increased mortality relative to the non-treated control for bollworms enclosed in bloom tags (F = 15.12; df = 3, 19.6; p < 0.01), but no differences were observed among insecticides (Fig. 4). Mean mortality provided by chlorantraniliprole and the methoxy-fenozide plus spinetoram premix ranged from 49 to 67%, whereas mean mortality in the non-treated control did not exceed 10%.



Figure 4. Bollworm mortality on closed cotton blooms after treatment with chlorantraniliprole at 0.053 kg ai ha⁻¹, chlorantraniliprole at 0.075 kg ai ha⁻¹, or methoxyfenozide + spinetoram at 0.21 kg ai ha⁻¹. Percent mortality \pm SEM 3 d after application. Stoneville, MS, 2019. Means separation was conducted with normalized (log10) data. Data (%) presented are not normalized. Columns containing the same letter are not significantly different (Tukey-Kramer HSD test, $\alpha = 0.05$).

DISCUSSION

Bt Floral Tissue Bioassay. Bollworm mortality was observed following feeding in flowers of Bt cotton, and this did not vary whether the insects originated from Bt or non-Bt corn. However, larvae were less susceptible to Bt in Bollgard II flowers than in Bollgard 3 flowers. Bollworm mortality in Bollgard II flowers has decreased since Gore et al. (2001) found that the combination of these proteins in floral structures provided higher levels of mortality when compared with Bollgard (single Bt gene). Mortality rates observed on flower anthers (37%) and petals (64%) by Gore et al. (2001) were overall higher than what was observed in this study, even though the methodology was nearly identical. Larval mortality has declined in Bollgard II flowers with a current mortality rate of 16%, in this study. This can likely be attributed to an increase in levels of bollworm resistance to Cry1Ac and Cry2Ab (Kerns et al., 2018; Reisig et al., 2018), but we cannot rule out varietal differences.

Based on larval size and mortality observed in this study, Cry1Ac and Cry2Ab still provide some insecticidal activity, but survival of larvae in Bollgard II flowers was frequent. Previously, larval lengths were measured to determine the effects Bt proteins had on larval development, and the use of Cry1Ac caused larvae to be smaller than larvae fed on non-Bt floral tissue (Stewart et al., 2001). Cotton varieties that expressed Cry2Ab with Cry1Ac led to an even greater reduction in bollworm larval length. The sublethal effects of Bt crystalline proteins were still observed for bollworm populations in the current study based on larval weights, suggesting that resistance is not complete.

Larvae fed on Bollgard II flowers in this study weighed less than larvae fed on non-Bt flowers after a 3-d feeding period. This is possibly the result of selective feeding on specific floral components (Gore et al., 2005). Once larvae were placed onto meridic diet, larval weight gain did not differ between larvae that previously fed on Bollgard II and non-Bt flowers, suggesting that those that survived were able to complete development at a normal rate after removal from Bt-expressing tissues. Reduced feeding by bollworm neonates likely occurs in Bollgard II flowers because they are actively searching for tissues with low expression as observed by Gore et al. (2001), but this slowed their development resulting in smaller larvae. That larvae were able to recover and develop normally after being placed on meridic diet suggests that much of the stunting that occurs on Bt cotton is likely due to avoidance rather than insecticidal poisoning.

The incorporation of Vip3A into Bt cotton varieties has resulted in greater levels of mortality and sublethal insecticidal effects on bollworm larval development compared with Bollgard II (Yang et al., 2022). The novel mode of action (Lee et al., 2003) is provided by the vegetative insecticidal protein in combination with Cry proteins. Reduced larval weight on Bollgard 3 compared to Bollgard II cotton is presumably due to the novel mode of action of Vip3A causing greater toxicity to bollworm larvae. However, similar weight gain was observed on clean diet after exposure to either of the two Bt varieties, so the reduced weight gain while on the Bt flowers could be a result of avoidance and selective feeding on floral structures with lower levels of protein expression prior to placement on meridic diet.

Cotton expressing Cry1Ac, Cry2Ab, and Vip3A is likely to provide better control of bollworm populations that become established in white flowers. However, the observed mortality rate of 43% was not optimal in the current study after a 3-d period. Currently, limited data are available on the variation in spatial expression of Vip3A among different structures in cotton. However, mortality observed following feeding in Bollgard 3 flowers was only slightly higher than mortality levels observed previously on Bollgard II cotton (Gore et al., 2001), and

suggests similarities in toxicity to Bollgard II previously observed by Greenplate (1999) and Adamczyk et al. (2001). This observation might be an indication that Bollgard 3 flowers are a possible point of vulnerability for the Vip3A technology, similar to what was observed for earlier Bt cotton technologies (Gore et al., 2001). Bollworm survival and subsequent plant damage will likely increase in cotton expressing Vip3A over time, and this could be directly related to greater survival in flower anthers relative to other plant parts. Larvae that are capable of survival in Bollgard 3 flowers might move to another bloom or bloom tag as a secondary feeding site before feeding on fruit, where mortality of neonates is generally greater, as observed in previous research (Godbold et al., 2022). Once larvae increase in size, expression in squares and bolls might not be sufficient for control.

The widespread use of Bt corn expressing Vip3A could have a major impact on the longevity of Vip3A's ability to control bollworm in cotton. Yang et al. (2019) suggested that decreases in mortality and less severe sublethal effects can occur when larvae are exposed to the Vip3A protein, if the previous generation was also exposed to Vip3A. Because early bollworm populations can be found in corn (Stadelbacher, 1980), declines in efficacy could occur if Vip3A becomes a multi-crop tool for bollworm management. Although no differences were observed among colonies collected from Bt corn and non-Bt corn in the currently study, selection for resistance in Vip3A field corn could lead to greater levels of survival in Vip3A cotton in the future.

Insecticide Bioassay. Insecticides are commonly used in non-Bt cotton and as supplemental control in Bollgard II cotton. Previously, Bollgard II cotton required less supplemental control due to the toxicity of Cry1Ac and Cry2Ab to bollworm (Gore et al., 2008; Reisig et al., 2018). However, due to increased levels of resistance, the use of insecticides has become more frequent to prevent yield losses (Gore et al., 2008; Reisig et al., 2018). Insecticides including methoxyfenozide plus spinetoram or chlorantraniliprole provided significant control of bollworms in dried flowers. However, the level of control observed after 3 d was much lower compared with trials in soybeans where no larvae were observed 4 d after application of methoxyfenozide plus spinetoram or chlorantraniliprole (Cato et al., 2018). Based on the control provided by these insecticides when insects were exposed to the direct spray, floral components could be a factor that hinders insecticidal activity in cotton. This is the likely reason for having a maximum of 67% initial (3 d) control of bollworm in bloom tags with foliar insecticides. Further delay in insecticidal applications to bloom tags (> 1 d after white flower) should be evaluated for the potential effects of greater floral constriction on the control efficacy of insecticides.

After 3 d, many surviving larvae were observed still feeding within the floral structure or on the tops of bolls, underlying the floral base, whereas few were observed feeding inside the boll or outside of the entire fruiting form. Once inside the boll, larvae can remain for an extended period. This common larval behavior will allow larvae to feed without contacting lethal concentrations of insecticides (Farrar and Bradley, 1984). Residual efficacy of insecticides is likely to play an important role in controlling bollworms feeding under bloom tags or in other protected areas. Results from this research demonstrate the importance of floral structures in contributing to bollworm survival in Bt cottons and following foliar insecticide applications. Larvae exposed to sublethal doses of Bt proteins and foliar insecticides could play a vital role in the development of resistance to these toxicants.

DISCLAIMER

Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply approval or recommendation of the product to the exclusion of others that may be suitable.

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REFERENCES

Abd-Elghafar. S.F., C.O. Knowles, and M.L. Wall. 1993. Pyrethroid resistance in two field strains of *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Econ. Entomol. 86(6):1651– 1655. https://doi.org/10.1093/jee/86.6.1651

- Adamczyk Jr., J.J., L.C. Adams, and D.D. Hardee 2001. Field efficacy and seasonal expression profiles for terminal leaves of single and double *Bacillus thuringiensis* toxin cotton genotypes. J. Econ. Entomol. 94(6):1589–1593. https://doi.org/10.1603/0022-0493-94.6.1589
- Baker, J. 1982. Selective effects of insecticides on withinspecies variation: the lessons to be learnt when considering the environmental effects of pollutants. Agric. Environ. 7(2):187–198. https://doi.org/10.1016/0304-1131(82)90007-8
- Betz, F.S., B.G. Hammond, and R.L. Fuchs. 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Regul. Toxicol. Pharmacol. 32(2):156–173. https://doi.org/10.1006/rtph.2000.1426
- Braswell, L.R., D.D. Reisig, C.E. Sorenson, and G.D. Collins. 2019. *Helicoverpa zea* (Lepidoptera: Noctuidae) oviposition and larval vertical distribution in Bt cotton under different levels of nitrogen and irrigation. J. Econ. Entomol. 112(3):1237–1250. https://doi.org/10.1093/jee/ toz023
- Calvert, G.M., J. Karnik, L. Mehler, J. Beckman, B. Morrissey, J. Sievert, R. Barrett, M. Lackovic, L. Mabee, A. Schwartz, Y. Mitchell, and S. Moraga-McHaley. 2008. Acute pesticide poisoning among agricultural workers in the United States, 1998-2005. Am. J. Ind. Med. 51(12):883–898. https://doi.org/10.1002/ajim.20623
- Cato, A., G. Lorenz, N. Taillon, A. Plummer, J. Black, and B.C. Thrash. 2018. Efficacy of selected insecticides for control of lepidopterous pests in soybean, 2016. Arthropod Manage. Tests. 43(1):1–2. https://doi.org/10.1093/ amt/tsy087
- Clower, D.F. 1980. Changes in Heliothis spp. attacking cotton in recent years and how they affected control. p. 139–141 *In* Proc. Beltwide Cotton Prod. Res. Conf., St. Louis, MO. 6-10 Jan. 1980. Natl. Cotton Counc. Am., Memphis, TN.
- Dively, G.P., P.D. Venugopal, and C. Finkenbinder. 2016. Field-evolved resistance in corn earworm to Cry proteins expressed by transgenic sweet corn. Plos One: 11(12):e0169115. https://doi.org/10.1371/journal. pone.0169115
- Farrar Jr., R.R., and J.R. Bradley Jr. 1984. Effects of corolla retention in cotton on *Heliothis* spp. (Lepidoptera: Noctuidae) larval numbers and damage. J. Econ. Entomol. 77(6):1470–1472. https://doi.org/10.1093/jee/77.6.1470
- Farrar Jr., R.R., and J.R. Bradley Jr. 1985. Effects of withinplant distribution of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) eggs and larvae on larval development and survival on cotton. J. Econ. Entomol. 78(6):1233–1237. https://doi.org/10.1093/jee/78.6.1233

Fleming, D., F. Musser, D. Reisig, J. Greene, S. Taylor, M. Parajulee, G. Lorenz, A. Catchot, J. Gore, D. Kerns, S. Stewart, D. Boykin, M. Caprio and N. Little. 2018. Effects of transgenic *Bacillus thuringiensis* cotton on insecticide use, heliothine counts, plant damage, and cotton yield: a meta-analysis, 1996-2015. Plos One 13(7):e0200131. https://doi.org/10.1371/journal. pone.0200131

Gianessi, L.P., and J.E. Carpenter. 1999. Agricultural Biotechnology: Insect control benefits. National Center for Food and Agricultural Policy, Washington, D.C.

Godbold, R.E., W.D. Crow, A.L. Catchot, J. Gore, D.R. Cook, D.M. Dodds, F.M. Musser, and N.S. Little. 2022. Feeding behavior and fruiting form damage by bollworm (Lepidoptera: Noctuidae) in Bt cotton. J. Econ. Entomol. 115(1):160–167. https://doi.org/10.1093/jee/toab220

Gore, J., B.R. Leonard, and J.J. Adamczyk. 2001. Bollworm (Lepidoptera: Noctuidae) survival on 'Bollgard' and 'Bollgard II' cotton flower bud and flower components. J. Econ. Entomol. 94(6):1445–1451. https://doi. org/10.1603/0022-0493-94.6.1445

Gore, J., B.R. Leonard, G.E. Church, and D.R. Cook. 2002. Behavior of bollworm (Lepidoptera: Noctuidae) larvae on genetically engineered cotton. J. Econ. Entomol. 95(4):763–769. https://doi.org/10.1603/0022-0493-95.4.763

Gore, J., J.J. Adamczyk Jr., and C.A. Blanco. 2005. Selective feeding of tobacco budworm and bollworm (Lepidoptera: Noctuidae) on meridic diet with different concentrations of *Bacillus thuringiensis* proteins. J. Econ. Entomol. 98(1):88–94. https://doi.org/10.1093/jee/98.1.88

Gore, J., J.J. Adamczyk Jr., A. Catchot, and R. Jackson. 2008. Yield response of dual-toxin Bt cotton to *Helicoverpa* zea infestations. J. Econ. Entomol. 101(5):1594–1599. https://doi.org/10.1093/jee/101.5.1594

Graves, J.B., D.F. Clower, and J.R. Bradley Jr. 1967. Resistance of the tobacco budworm to several insecticides in Louisiana. J. Econ. Entomol. 60(3):887–888. https://doi. org/10.1093/jee/60.3.887

Graves, J.B., J.S. Roussel, and J.R. Phillips. 1963. Resistance to some chlorinated hydrocarbon insecticides in the bollworm, *Heliothis zea*. J. Econ. Entomol. 56(4):442–444. https://doi.org/10.1093/jee/56.4.442

Greenplate, J.T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. J. Econ. Entomol. 92(6):1377–1383. https://doi.org/10.1093/jee/92.6.1377

Gupta, M., H. Kumar, and S. Kaur. 2021. Vegetative insecticidal protein (Vip): a potential contender from *Bacillus thuringiensis* for efficient management of various detrimental agricultural pests. Front. Microbiol. 12:659736. https://doi.org/10.3389/fmicb.2021.659736 Ivy, E.E., and A.L. Scales. 1954. Are cotton insects becoming resistant to insecticides? J. Econ. Entomol. 47(6):981– 984. https://doi.org/10.1093/jee/47.6.981

Jacobson, A., R. Foster, C. Krupke, W. Hutchison, B. Pittendrigh, and R. Weinzierl. 2009. Resistance to pyrethroid insecticides in *Helicoverpa zea* (Lepidoptera: Noctuidae) in Indiana and Illinois. J. Econ. Entomol. 102(6):2289– 2295. https://doi.org/10.1603/029.102.0634

Kenward, M.G., and J.H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53(3):983–997. https://doi. org/10.2307/2533558

Kerns, D.L., F. Yang, G.M. Lorenz, J. Gore, A.L. Catchot,
S.D. Stewart, S.A. Brown, D.R. Cook, and N. Seiter.
2018. Value of Bt technology for bollworm management.
p. 805–809 *In* Proc. Beltwide Cotton Conf., San Antonio,
TX. 3-5 Jan. 2017. Natl. Cotton Counc. Am., Memphis,
TN.

Lee, M.K., F.S. Walters, H. Hart, N. Palekar, and J.S. Chen. 2003. The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab δ-endotoxin. Appl. Environ. Microbiol. 69(8):4648–4657. https://doi.org/10.1128/ AEM.69.8.4648-4657.2003

Mauney, J.R. 2012. Anatomy and morphology of fruiting forms. p. 1–11 *In* D. M. Oosterhuis and J.T. Cothren (ed.), Flowering and Fruiting in Cotton. William C. Robertson, Cordova, TN.

Naranjo, S.E. 2011. Impacts of Bt transgenic cotton on integrated pest management. J. Agric. Food Chem. 59(11):5842–5851. https://doi.org/10.1021/jf102939c

Pietrantonio, P.V., T.A. Junek, R. Parker, D. Mott, K. Siders, N. Troxclair, J. Vargas-Camplis, J.K. Westbrook, and V.A. Vassiliou. 2007. Detection and evolution of resistance to the pyrethroid cypermethrin in *Helicoverpa zea* (Lepidoptera: Noctuidae) populations in Texas. Enviro. Entomol. 36(5):1174–1188. https://doi. org/10.1603/0046-225X(2007)36[1174:DAEORT]2.0. CO;2

Plapp Jr., F.W. 1971. Insecticide resistance in *Heliothis*: tolerance in larvae of *H. virescens* as compared with *H. zea* to organophosphate insecticides. J. Econ. Entomol. 64(5):999–1002. https://doi.org/10.1093/jee/64.5.999

Reisig, D.D., A.S. Huseth, J.S. Bacheler, M. Aghaee, L. Braswell, H.J. Burrack, K. Flanders, J.K. Greene, D.A. Herbert, A. Jacobson, S.V. Paula-Moraes, P. Roberts, and S.V. Taylor. 2018. Long-term empirical and observational evidence of practical *Helicoverpa zea* resistance to cotton with pyramided Bt toxins. J. Econ. Entomol. 111(4):1824–1833. https://doi.org/10.1093/jee/toy106

- Smith, R.H. 1997. An extension entomologist's 1996 observations of Bollgard Bt technology. p. 856–858 *In* Proc.
 Beltwide Cotton Conf., New Orleans, LA. 7-10 Jan.
 1997. Natl. Cotton Counc. Am., Memphis, TN.
- Snodgrass, G.L., and W.P. Scott. 2000. Seasonal changes in pyrethroid resistance in tarnished plant bug (Heteroptera: Miridae) populations during a three-year period in the delta area of Arkansas, Louisiana, and Mississippi. J. Econ. Entomol. 93(2):441–446. https://doi. org/10.1603/0022-0493-93.2.441
- Sparks, T.C. 1981. Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America. Bull. Entomol. Soc. Amer. 27:186–192.
- Stadelbacher, E. 1980. Oviposition preference of the bollworm for species of early-season host plants in the delta of Mississippi. Environ. Entomol. 9(5):542–545. https:// doi.org/10.1093/ee/9.5.542
- Stadelbacher, E.A., G.L. Snodgrass, and G.W. Elzen. 1990. Resistance to cypermethrin in first generation adult bollworm and tobacco budworm (Lepidoptera: Noctuidae) populations collected as larvae on wild geranium, and in the second and third larval generations. J. Econ. Entomol. 83(4):1207–1210. https://doi.org/10.1093/jee/83.4.1207
- Staetz, C.A. 1985. Susceptibility of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) to permethrin from across the cotton belt: a five-year study. J. Econ. Entomol.78(3):505–510. https://doi.org/10.1093/jee/78.3.505
- Stewart, S.D., J.J. Adamczyk Jr., K.S. Knighten, and F.M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival of noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94(3):752–760. https://doi. org/10.1603/0022-0493-94.3.752
- Tukey, J.W. 1953. The problem of multiple comparisons. Dept. of Statistics, Princeton University, NJ.
- Wilkins, T.A., K. Rajasekaran, and D.M. Anderson. 2000. Cotton biotechnology. Crit. Rev. Plant Sci. 19(6):511– 550. https://doi.org/10.1080/07352680091139286
- Wu, M., G. Li, X. Chen, J. Liu, M. Liu, C. Jiang, and Z. Li. 2018. Rational dose of insecticide chlorantraniliprole displays a transient impact on the microbial metabolic functions and bacterial community in a silty-loam paddy soil. Sci. Total Environ. 616-617:236–244. https://doi. org/10.1016/j.scitotenv.2017.11.012
- Yang, F., J.C.S. González, J. Williams, D.C. Cook, R.T. Gilreath, and D.L. Kerns. 2019. Occurrence and ear damage of *Helicoverpa zea* on transgenic *Bacillus thuringiensis* maize in the field in Texas, U.S. and its susceptibility to Vip3A protein. Toxins. 11(2):102. https://doi. org/10.3390%2Ftoxins11020102

- Yang, F., D.L. Kerns, N. Little, S.A. Brown, S.D. Stewart, A.L. Catchot, D.R. Cook, J. Gore, W.D. Crow, G.M. Lorenz, T. Towles, and B.E. Tabashnik. 2022. Practical resistance to Cry toxins and efficacy of Vip3Aa in Bt cotton against *Helicoverpa zea*. Pest Manag. Sci. https://doi. org/10.1002/ps.7142 (verified 27 Oct. 2022).
- Zar, J.H. 1999. Biostatistical Analysis. Prentice Hall, Upper Saddle River, NJ.