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Contribution of *Bacillus thuringiensis* cotton cultivars and insecticides to a fall armyworm, *Spodoptera frugiperda* (J. E. Smith), (Lepidoptera : Noctuidae) management strategy

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**CONTRIBUTION OF *BACILLUS THURINGIENSIS* COTTON
CULTIVARS AND INSECTICIDES TO A FALL ARMYWORM,
SPODOPTERA FRUGIPERDA (J. E. SMITH), (LEPIDOPTERA:
NOCTUIDAE) MANAGEMENT STRATEGY**

A Dissertation

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

in

The Department of Entomology

by
Jarrod T. Hardke
B.S. University of Arkansas, 2006
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ABSTRACT

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), response to cottons, *Gossypium hirsutum* L., expressing *Bacillus thuringiensis* (Bt) insecticidal proteins, Cry1Ac (Bollgard[®]), Cry1Ac + Cry2Ab (Bollgard II[®]), and Cry1Ac + Cry1F (WideStrike[™]), was evaluated in field and laboratory experiments. In field trials, larvae that were infested on selected fruiting forms (squares, white flowers, and bolls) of WideStrike[™] plants had lower survivorship and caused less injury than larvae on non-Bt plants, regardless of fruiting structures. Bollgard[®] and Bollgard II[®] plants produced no consistent negative effects on fall armyworm survivorship and injury. In no-choice laboratory assays, Bollgard II[®] and WideStrike[™] cotton tissue reduced fall armyworm larval development and survivorship compared to those larvae offered non-Bt tissue. Fall armyworm preference for oviposition sites on non-Bt and Bt-expressing cotton plants was evaluated by releasing adults into isolation cages containing plants of a single cotton line. The distribution of egg masses on non-Bt, Bollgard[®], Bollgard II[®], and WideStrike[™] cotton plants was similar with the majority of egg clusters observed on the abaxial (underside) leaf surfaces. The field performance of selected novel and standard insecticides was evaluated against fall armyworm in conventional non-Bt cotton, sprayed with recommended (full) rates of products, and in Bollgard II[®] cotton, sprayed with reduced (one-half) rates of the same products. Insecticide-treated terminal leaves and bolls were removed from plants in a field environment, placed in plastic dishes and infested with a single third instar. Reducing insecticide rates on Bollgard II[®] cotton did not negatively affect efficacy of any insecticide compared to efficacy of full rates applied to conventional non-Bt cotton. These results show differences between the currently available Bt cotton technologies in their performance against fall armyworm larvae. This information should be used by the cotton industry in the selection of the most appropriate Bt

traits if fall armyworm is considered a prevalent pest. Furthermore, opportunities to reduce insecticide rates without sacrificing satisfactory efficacy against fall armyworm on Bollgard II® plants could reduce chemical control costs. To better characterize fall armyworm identification and injury symptomology, descriptions and photographs were compiled in a manner that should be useful to cotton pest managers and producers.

CHAPTER 1

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), has been documented as a pest of cotton, *Gossypium hirsutum* (L.), since 1912 (Dew 1913), occurring sporadically due to its migratory behavior. This species does not enter diapause, so it migrates each year from warmer climates such as southern Florida, the Caribbean Islands, southern Texas, Mexico, and coastal areas of southern Georgia, Alabama, Mississippi, and Louisiana, across the U.S. annually (Luginbill 1928, Sparks 1979, Knipling 1980, Ashley et al. 1989, Adamczyk 1998). Fall armyworm populations spread from the source areas each spring in a northward and westward direction at an estimated rate of 300 miles per generation, with an extended geographic range from the Rocky Mountains to Canada (Pair et al. 1986, Ashley et al. 1989). Fall armyworm movement each year generally creates sporadic problems across multiple crops. Damage caused by outbreaks of this pest can be unpredictable, damaging a range of plant structures from vegetative to reproductive, and this species has the potential to cause devastating crop losses.

The fall armyworm was the seventh most damaging pest of cotton in the U.S. during the 2006 growing season, and the fifth most damaging pest in 2009. The fall armyworm reduced yield in the U.S. by 0.069% in 2006, while infesting approximately 3.92 million acres of cotton. In 2009, fall armyworm reduced yield in the U.S. by 0.113%, while infesting approximately 1.84 million acres of cotton. This reduction in yield equates to a loss of 24,991 cotton bales from fall armyworm in 2006, compared to 20,238 bales lost in 2009 (Williams 2007a, Williams 2010a).

Across the cotton belt, nine states, including Tennessee (0.299%), Mississippi (0.215%), Arkansas (0.169%), Louisiana (0.095%), Alabama (0.073%), Georgia (0.045%), Texas (0.018%), Florida (0.006%) and New Mexico (0.001%) reported losses due to fall armyworm in

2006, whereas eight states (Arizona, California, Kansas, Missouri, North Carolina, Oklahoma, South Carolina, and Virginia) reported no losses and three states (California, Kansas and Virginia) reported no infestations (Williams 2007a). In 2009, nine states reported losses to fall armyworm, including Florida (1.35%), South Carolina (0.9%), Alabama (0.73%), Georgia (0.3%), North Carolina (0.083%), Texas (0.044%), Tennessee (0.03%), Louisiana (0.02%), and Arkansas (0.019%) (Williams 2010a). Eight states (Arizona, California, Kansas, Mississippi, Missouri, New Mexico, Oklahoma, and Virginia) reported no losses and five states (California, Kansas, New Mexico, Oklahoma, and Virginia) reported no infested acres (Williams 2010a).

In 2006, Louisiana reported 281,700 of 626,000 planted acres were infested with fall armyworm. Of those acres, 93,900 were treated. Cotton yield loss due to fall armyworm was 0.09%, or 1,601 bales (Williams 2007b). In 2009, Louisiana reported 45,600 of the 228,000 planted acres, were infested with fall armyworm. Of those acres, 22,800 were treated. Cotton yield loss due to fall armyworm was 0.02%, with 124 bales lost (Williams 2010b).

Traditional Chemical Control Strategies Used Against Fall Armyworm

The bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.), collectively known as the heliothine complex, are important cotton pests. Insecticides have long been prominent in controlling more common pests, such as the bollworm and the tobacco budworm, but the majority of these compounds have been largely ineffective in controlling the fall armyworm. The behavioral habits of the fall armyworm make it particularly difficult to control with insecticides. This insect disperses low in the plant canopy, making it safe from popular present-day foliar insecticide applications (Adamczyk et al. 1997, Adamczyk 1998). Currently, the recommended insecticides to control fall armyworm include pyrethroids (lambda-cyhalothrin, zeta-cypermethrin, etc.), carbamates (thiodicarb), spinosyns (spinosad), and insect

growth regulators (benzoylureas [novaluron] and diacylhydrazines [methoxyfenozide]) (Baldwin et al. 2010, IRAC Mode of Action Working Group 2010). The rates of recommended products to control fall armyworm are equal to or above the recommended rates to control bollworm and tobacco budworm (Baldwin et al. 2010). However, based upon available information in the Arthropod Management Tests (Entomological Society of America, www.entsoc.org), limited insecticide efficacy data for control of fall armyworm exists, particularly from field experiments. In addition, there are newly registered insecticides on cotton for which no data on fall armyworm control exists.

Introduction of Genetically Engineered Cotton

The first transgenic *Bacillus thuringiensis* (Berliner), (Bt), cotton became available in the U.S. in 1996 (Adamczyk et al. 2000, Adamczyk et al. 2001a, Adamczyk and Gore 2004, Naranjo et al. 2008). In 1995, prior to commercialization of Bt cotton, 15.69 million acres of upland cotton was planted across the U.S. (1.06 million in Louisiana) (Williams 1996). By 2000, Bt cotton accounted for 34% (5.22 million acres) of U.S. cotton acreage (of 15.36 million total acres) (Williams 2001). In Louisiana during 2000, Bt cotton accounted for 80% (0.57 million acres) of the total 0.71 million planted acres. In 2007, Bt cotton acreage had increased to 67% (7.1 million acres) of U.S. cotton acreage (10.53 million total acres) (Williams 2008). For Louisiana in 2007, Bt cotton accounted for 91% of the total 0.33 million acres planted.

As of 2006, the U.S. has adopted a higher percentage (52%) of genetically modified (GM) crops (combined herbicide tolerance and insect resistance traits) than any other country in the world, followed by Argentina (18%) and Brazil (12%) (Brookes and Barfoot 2008). Global adoption of GM cotton (combined herbicide tolerance and insect resistance traits) has increased from 0 acres in 1995 to \approx 60 million acres in 2006, with Bt cotton accounting for 11% of that

total (Figure 1.1). In countries planting insect resistant cotton, Bt cotton accounted for 2.1 million acres of the 48.9 million acres planted worldwide. By 2006, Bt cotton had grown to account for 28.6 million acres of the 56 million acres planted around the world.

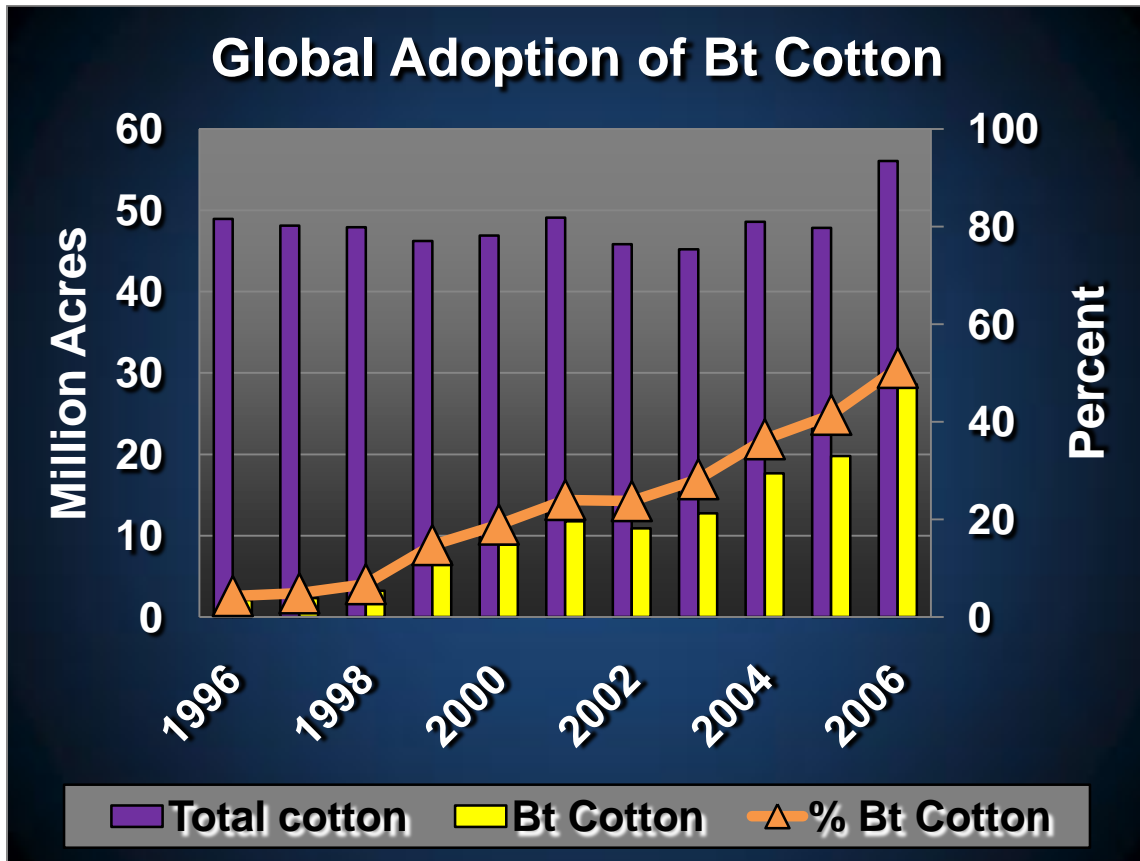


Figure 1.1. Global adoption of Bt cotton in million acres and percent of total. Compiled from Brooks and Barfoot (2008)

Bollgard[®] cotton, produced by Monsanto, contains a gene which encodes for the production of an insecticidal crystal protein, Cry1Ac δ -endotoxin from Bt (Adamczyk and Gore 2004, Jackson et al. 2005, Leonard et al. 2006). Lepidopteran pests, such as the tobacco budworm, are particularly susceptible to the Cry1Ac endotoxin (Adamczyk and Gore 2004). The development of resistance to various classes of insecticides by the tobacco budworm (Baldwin et al. 2010) greatly facilitated the need for this technology. The bollworm, as well as other more sporadic Lepidopteran pests, is considerably more tolerant to the Cry1Ac endotoxin

than the tobacco budworm (Stewart et al. 2000). The lack of bollworm control with Bollgard[®] led to the development of new Bt technologies which express two proteins and provide adequate control of bollworm and other Lepidopteran pests. In 2002, Bollgard II[®] cotton was commercially released by Monsanto, Co. These varieties produce the Bt endotoxins Cry1Ac and Cry2Ab. Expression of Cry1Ac in Bollgard II[®] is similar to that in Bollgard[®]. In comparison, Cry2Ab expression in Bollgard II[®] has been found to be 3-5 times higher than Cry1Ac expression (Jackson et al. 2003, Adamczyk and Gore 2004, Akin et al. 2004, Jackson et al. 2005, Adamczyk and Mahaffey 2007).

In 2005, Dow AgroSciences released a similar technology in their WideStrike[™] varieties. WideStrike[™] varieties produce the same Cry1Ac Bt endotoxin in both Bollgard[®] and Bollgard II[®], but also produce a Cry1F endotoxin (Adamczyk and Gore 2004, Adamczyk and Mahaffey 2007, Naranjo et al. 2008). Bollgard II[®] and WideStrike[™] currently are the only commercially available cotton varieties which produce two endotoxins. With the Cry1F endotoxin in WideStrike[™], this technology provides control of secondary Lepidopteran pests such as cabbage looper, *Trichoplusia ni* (Hübner); soybean looper, *Pseudoplusia includens* (Walker); saltmarsh caterpillar, *Estigmene acrea* (Drury); and European corn borer, *Ostrinia nubilalis* Hübner; as well as control of heliothines (Haile et al. 2004, Siebert et al. 2007).

In addition to these available technologies, Syngenta Crop Protection has been developing a novel Bt exotoxin (Vip3A) (Adamczyk and Gore 2004, Adamczyk and Mahaffey 2007). This vegetative insecticidal protein (Vip) is unlike other Bt proteins currently available, both in structure as well as in mode of action (Estruch et al. 1996, McCaffery et al. 2006). This protein has now been licensed to other companies for use in their Bt cotton lines and, in the future, it will be utilized in Bollgard III and WideStrike[™] advanced lines.

Bayer CropScience is also in the process of developing varieties within their FiberMax lines that express Bt proteins. Their technology, still in the early stages of development, also will utilize dual gene Bt toxin technology (Cry1Ab and Cry2Ab). This technology, known as TwinLink, would provide an additional, and unique, combination of Bt proteins to cotton growers. The continued development and use of diverse combinations of proteins will help to ensure the use of this technology.

While fall armyworm is currently not considered a primary target pest of Bt cottons, it has the potential to become an important future pest. History tells us that insects will fill niches in the absence of broad spectrum control efforts. This is evident in the rise of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), from a secondary to a primary pest. The inception of Bt cotton led to a reduction in incidental control with insecticide sprays for lepidopteran pests. Additionally, it is important that each of these technologies be characterized against our pest spectrum. With this information we will be able to identify the strengths and weaknesses of the technologies. This information also will help to improve integrated pest management tactics, as scouting can focus on those pests which are less susceptible to Bt proteins. Also, no current literature exists which provides graphic descriptions of fall armyworm injury to cotton, particularly those plants which express Bt proteins.

Objectives

The following objectives were proposed:

- I. To evaluate fall armyworm development and survivorship, and characterize damage on transgenic Bt cotton cultivars, including Bollgard[®], Bollgard II[®], and WideStrike[™], as well as conventional non-Bt varieties.

- II. To examine behavioral effects of transgenic and conventional cotton cultivars on fall armyworm oviposition.
- III. To evaluate novel insecticide efficacy against fall armyworm larvae on transgenic and conventional cotton cultivars.
- IV. To describe the symptomology of fall armyworm injury on transgenic and conventional cotton cultivars with graphics and visual aids.

References Cited

- Adamczyk, Jr., J. J., J. W. Holloway, B. R. Leonard, and J. B. Graves. 1997.** Defining the period of boll susceptibility to fall armyworm injury in cotton, pp. 941-944. *In Proc. 1997 Beltwide Cotton Conf., New Orleans, LA. January 6-10, 1997.*
- Adamczyk, Jr., J. J. 1998.** Pest status of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), on conventional and transgenic cotton expressing the *Bacillus thuringiensis* Cry1A(c) δ -endotoxin: development, survival, and host-strain influence. Dissertation: Dept. of Entomology, Louisiana State University and Agricultural and Mechanical College.
- Adamczyk, Jr., J. J., L. C. Adams, and D. D. Hardee. 2000.** Quantification of Cry1A(c) δ -endotoxin in transgenic Bt cotton: correlating insect survival to different protein levels among plant parts and varieties, pp. 929-932. *In Proc. 2000 Beltwide Cotton Conf., San Antonio, TX. January 4-8, 2000.*
- Adamczyk, Jr., J. J., D. D. Hardee, L. C. Adams, and D. V. Sumerford. 2001a.** Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1A(c) δ -endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *J. Econ. Entomol.* 94: 284-290.
- Adamczyk, Jr., J. J. and J. Gore. 2004.** Laboratory and field performance of cotton containing Cry1Ac, Cry1F, and both Cry1Ac and Cry1F (WideStrike®) against beet armyworm and fall armyworm larvae (Lepidoptera: Noctuidae). *Florida Entomol.* 87: 427-432.
- Adamczyk, Jr., J. J. and J. S. Mahaffey. 2007.** Efficacy of Vip3A and Cry1Ab genotypes against various lepidopteran pests, pp. 1106-1113. *In Proc. 2007 Beltwide Cotton Conf., New Orleans, LA. January 9-12, 2007.*
- Akin, D. S., M. B. Layton, S. D. Stewart, and J. J. Adamczyk, Jr. 2004.** Temporal expression profiles and bioactivity of single (Bollgard®) and dual-toxin (Bollgard II®)

- Bt cotton, pp., 1422-1429. *In Proc. 2004 Beltwide Cotton Conf.*, San Antonio, TX. January 5-9, 2004.
- Ashley, T. R., B. R. Wiseman, F. M. Davis, and K. L. Andrews. 1989.** The fall armyworm: a bibliography. *Florida Entomol.* 72: 152-202.
- Baldwin, J. L., L. D. Foil, M. L. Grodner, A. Hammond, G. Henderson, N. Hummel, S. Johnson, R. Leonard, A. Morgan, D. K. Pollet, J. Pyzner, T. E. Reagan, D. Reed, D. Ring, R. N. Story, and M. Stout. 2010.** 2010 Louisiana Insect Pest Management Guide. LSU AgCenter [Online]. Available at http://www.lsuagcenter.com/en/communications/publications/management_guides/Louisiana+Insect+Pest+Management+Guide.htm (verified 16 May 2011).
- Brookes, G. and P. Barfoot. 2008.** GM crops: global socio-economic and environmental impacts 1996-2006. PG Economics Ltd. 118 pp.
- Dew, J. A. 1913.** Fall army worm. *J. Econ. Entomol.* 6: 361-366.
- Estruch, J. J., G. W. Warren, M. A. Mullins, G. J. Nye, J. A. Craig, and M. G. Koziel. 1996.** Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proc. Natl. Acad. Sci.* 93: 5389-5394.
- Haile, F. J., L. B. Braxton, E. A. Flora, B. Haygood, R. M. Huckaba, J. W. Pellow, V. B. Langston, R. B. Lassiter, J. M. Richardson, and J. S. Richburg. 2004.** Efficacy of WideStrike cotton against non-heliothine Lepidopteran insects. *In Proc. 2004 Beltwide Cotton Conf.*, San Antonio, TX. January 5-9, 2004.
- IRAC International MoA Working Group. 2010.** IRAC MoA classification scheme. Version 7.0 [Online]. Available at http://www.irc-online.org/wp-content/uploads/2009/09/MoA-classification_v7.0.4-5Oct10.pdf (verified Mar. 28, 2011).
- Jackson, R. E., J. R. Bradley, Jr., and J. W. Van Duyn. 2003.** Field performance of transgenic cottons expressing one or two *Bacillus thuringiensis* endotoxins against bollworm, *Helicoverpa zea* (Boddie). *J. Cotton Sci.* 7: 57-64.
- Jackson, R. E., J. R. Bradley, and J. W. Van Duyn. 2005.** Comparative efficacy of Bt technologies against bollworm in North Carolina, pp. 1373-1378. *In Proc. 2005 Beltwide Cotton Conf.*, New Orleans, LA. January 4-7, 2005.
- Knipling, E. F. 1980.** Regional management of the fall armyworm—a realistic approach? *Florida Entomol.* 63: 468-480.
- Leonard, B. R., K. V. Tindall, and K. D. Emfinger. 2006.** Fall armyworm survivorship and damage in Bollgard and Bollgard 2 cotton, pp. 1080-1084. *In Proc. 2006 Beltwide Cotton Conf.*, San Antonio, TX. January 3-6, 2006.

- Luginbill, P. 1928.** The fall armyworm. USDA Tech. Bull. No. 34.
- McCaffery, A., M. Caprio, R. Jackson, M. Marcus, T. Martin, D. Dickerson, D. Negrotto, D. O'Reilly, E. Chen, and M. Lee. 2006.** Effective IRM with the novel insecticidal protein Vip3A, pp. 1229-1235. *In Proc. 2006 Beltwide Cotton Conf., San Antonio, TX. January 3-6, 2006.*
- Naranjo, S. E., J. R. Ruberson, H. C. Sharma, L. Wilson, and K. Wu. 2008.** The present and future role of insect-resistant genetically modified cotton in IPM, pp. 159-194. *In J. Romeis, A. M. Shelton, and G. G. Kennedy (eds.) Integration of insect-resistant genetically modified crops within IPM programs. Progress in Bio. Control Vol. 5.*
- Pair, S. D., J. R. Raulston, A. N. Sparks, J. K. Westbrook, and G. K. Douce. 1986.** Fall armyworm distribution and population dynamics in the southeastern states. *Florida Entomol. 69: 468-487.*
- Siebert, M. W., L. B. Braxton, R. M. Huckaba, L. C. Walton, R. A. Haygood, R. B. Lassiter, F. J. Haile, and G. D. Thompson. 2007.** Field performance of Dow AgroScience's WideStrike™ insect protection against key Lepidopteran pests in the mid-south and southeastern U.S. *In Proc. 2007 Beltwide Cotton Conf., New Orleans, LA. January 9-12, 2007.*
- Sparks, A. N. 1979.** A review of the biology of the fall armyworm. *Florida Entomol. 62: 82-87.*
- Stewart, S. D., K. S. Knighten, and F. M. Davis. 2000.** Efficacy of Bt cotton expressing two insecticidal proteins of *Bacillus thuringiensis* Berliner on selected caterpillar pests, pp. 1043-1049. *In Proc. 2000 Beltwide Cotton Conf., San Antonio, TX. January 4-8, 2000.*
- Williams, M. R. 1996.** Cotton insect losses 1995, pp. 670-689. *In Proc. 1996 Beltwide Cotton Conf., Nashville, TN. January 9-12, 1996.*
- Williams, M. R. 2001.** Cotton insect losses 2000. *In Proc. 2001 Beltwide Cotton Conf., Anaheim, CA. January 9-13, 2001.*
- Williams, M. 2007a.** Cotton insect loss estimates – 2006, pp. 1027-1042. *In Proc. 2007 Beltwide Cotton Conf., New Orleans, LA. January 9-12, 2007.*
- Williams, M. 2007b.** Cotton insect losses – 2006, pp. 974-1026. *In Proc. 2007 Beltwide Cotton Conf., New Orleans, LA. January 9-12, 2007.*
- Williams, M. R. 2008.** Cotton insect losses – 2007, pp. 927-979. *In Proc. 2008 Beltwide Cotton Conf., Nashville, TN. January 8-11, 2008.*
- Williams, M. R. 2010a.** Cotton insect loss estimates – 2009, pp. 1099-1110. *In Proc. Beltwide Cotton Conf., New Orleans, LA. January 4-7, 2010.*

Williams, M. R. 2010b. Cotton insect losses – 2009, pp. 1029-1073. *In* Proc. 2010 Beltwide Cotton Conf., New Orleans, LA. January 4-7, 2010.

CHAPTER 2

REVIEW OF CURRENT LITERATURE

Introduction

In the United States (U.S.), upland cotton, *Gossypium hirsutum* (L.), was produced on an estimated 14.9 million acres (635,000 acres in Louisiana) in 2006, but acreage decreased significantly in subsequent years to 9.0 million acres (230,000 in Louisiana) in 2009. The average yield for upland cotton across the nation in 2006 was 811 lb lint/acre (952 lb lint/acre in Louisiana), while in 2008 average yield was 799 lb lint/acre (560 lb lint/acre in Louisiana) (Williams 2007b, Williams 2009, Williams 2010b). A number of arthropods are known to be pests of cotton throughout its life cycle, most notably thrips (multiple species), tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), heliothines, *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.), stink bugs (multiple species), fall armyworm, and spider mites, *Tetranychus* spp. Of these, fall armyworm is a sporadic, but economically important, pest of cotton in the Mid-South and Southeast U.S.

Fall Armyworm Biology

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a sporadic pest due to its migratory behavior. This species does not enter diapause, so it migrates each year from warmer climates such as southern Florida, the Caribbean islands, southern Texas, Mexico, and coastal areas of southern Georgia, Alabama, Mississippi, and Louisiana, across the U.S. annually (Luginbill 1928, Sparks 1979, Knipling 1980, Ashley et al. 1989). Fall armyworm populations spread from the source areas each spring in a northwesterly direction at an estimated rate of 300 miles per generation, with an extended geographical range from the Rocky Mountains to Canada (Pair et al. 1986, Ashley et al. 1989). Fall armyworm movement each year generally creates

sporadic problems across multiple crops. Damage caused by fall armyworm outbreaks can be unpredictable; damaging a range of plant structures from vegetative to reproductive, and this species has the potential to cause devastating crop losses.

The fall armyworm is classified in the order Lepidoptera and the family Noctuidae. The fall armyworm is a serious, albeit sporadic, pest of cotton and corn, *Zea mays* (L.), as well as many grass crops, across the Southern U.S. (Luttrell and Mink 1999, Jackson et al. 2007).

The fall armyworm has several generations per year. The life cycle consists of – egg, five-six instars, pupa, and adult. Completion of the life cycle usually takes about four weeks, but in cold weather can take as long as 12 weeks. Eggs are generally laid on the abaxial (underside) surface of leaves, but when the oviposition frequency in the area is high, females will deposit eggs on all plant structures (Luginbill 1928, Sparks 1979, Ali et al. 1989). Eggs, at times, will be covered with down (dense covering of scales and silken threads) from the moth. The eggs are laid in groups ranging in size from several eggs to > hundred and will usually hatch within four days under optimal conditions (Dew 1913, Luginbill 1928, Sparks 1979).

The most preferred location for oviposition is on leaves (95.9%) emerging directly from the main stem in the middle to lower portion of the plant canopy (Ali et al. 1989). On vegetative stage plants, 86.9% of egg masses are found between main stem nodes 0 and 5 (average number of nodes = 11). Later in the growing season, on reproductive stage plants, 97.4% of egg masses are found between nodes 0 and 10 (average number of nodes = 16). Once cotton has begun to bloom, 84.5% of egg masses are found between nodes 0 and 10 (average number of nodes = 18). Egg masses on cotton plants during mid-flowering are found between nodes 0 and 15 (average number of nodes = 21) 98.1% of the time. On cotton plants with mature bolls, 96.2% of egg masses are found between nodes 0 and 15 (average number of nodes = 23). Finally, on cotton

plants displaying open bolls, 96.1% of egg masses can be found between nodes 0 and 15 (average number of nodes = 25) (Ali et al. 1989).

Larvae pupate at a depth of one to three inches in the soil and remain there for about seven to ten days (Robinson 1999). The depth in the soil is dependent upon factors such as texture, moisture, and temperature of the soil (Sparks 1979). As moths emerge from the pupal stage, they migrate up to 300 miles before mating and ovipositing (Ashley et al. 1989, Robinson 1999). As many as 10 generations per year may occur in areas of the Southern U.S (Robinson 1999).

Fall Armyworm Description

The egg of the fall armyworm is “oblate-spheroidal”. Eggs are initially greenish gray in color, and become progressively darker with age. Twelve hours after deposition, eggs are generally brown, and appear nearly black just prior to larval eclosion (Luginbill 1928). When first instars eclose from eggs, they are off-white to yellow with black head capsules and have small black dots from which primary setae protrude. As larvae feed they darken in color and appear to be greenish (Luginbill 1928). Additional larval instars are similar in color to earlier instars just after molting, but typically darken in color just prior to molting to a new instar.

The three final instars are typically dark in color, with varying color patterns depending on their diet and other factors. The larva displays a prominent inverted “Y” on the head capsule. The head capsule is traditionally dark in color, ranging from brown to black. These later instars lack primary setae and are generally smooth (Oliver and Chapin 1981, Robinson 1999). Larvae may range from light green to brown to even appearing nearly black in color. Markings on the larvae can include a non-continuous white line in the mid-dorsal area, as well as yellow and red

“flecking” on the venter (abdomen). Fall armyworm larvae also possess teeth on their mandibles.

Fall armyworm adults or moths have a wing-span of about one and one-half inches. The upper portion of the forewings are a mottled dark gray in color, with a distinctive white spot near the dorsal tip, or apex, of the wing, while the lower portion of the forewings is a light gray to brown color. The hind wings are light gray to white in color. Male adults are often confused with yellow-striped armyworm, *Spodoptera ornithogalli* (Guenée), which have more contrast in shades. Female adults may also be confused at times with beet armyworm, *Spodoptera exigua* (Hübner). Fall armyworm moths also have filiform (threadlike) antennae. These moths are generally active at night which is common among Noctuids (Oliver and Chapin 1981, Robinson 1999).

Fall armyworm survivorship can vary significantly within a range of temperatures depending upon their life stage. At temperatures between 0°C and -2.5°C, mean percentage survival did not differ among life stages. However, at -5.0°C, -7.5°C, and -10.0°C, eggs and small larvae (L1-L3 stages) survived at significantly higher rates than large larvae (L4-L6 stages), pupae, adult males and adult females (Foster and Cherry 1987). Simmons reported that no pupae held at 10°C survived to the adult stage (Simmons 1993).

Fall Armyworm Strains

The behavior of fall armyworm is complicated due to the existence of two separate, though morphologically identical, strains. Pashley (1986) initially classified fall armyworm populations as being composed of sibling species, or strains. These strains are referred to as “host specific” as a result of their host plant preferences. They are commonly referred to as the rice-strain (R-strain) and the corn-strain (C-strain) (Quisenberry 1991, Nagoshi and Meagher

2004). Fall armyworm of the R-strain prefer rice, *Oryza sativa* (L.), and bermudagrass, *Cynodon dactylon* (L.) Pers., whereas the C-strain prefers field and sweet corn. These differences in host preference can have significant effects on fall armyworm development (Pashley et al. 1995).

Further differences in genetic differentiation were detected at the population level and between the two fall armyworm strains using mitochondrial DNA (Lewter et al. 2006). The identity of fall armyworm strains has also been confirmed through the use of DNA sequencing (Levy et al. 2002). Several possibilities have been discussed as to the taxa represented by these strains. They may be biotypes in which genetic differences are due to a selectively-mediated polymorphism within a single random-mating species. The strains may represent host races in the initial stages of speciation in which interbreeding is reduced to host preferences. Lastly, they may be sibling species that are either capable of hybridizing to a limited degree or completely reproductively isolated. Current studies do not support the two strains as biotypes. In addition, life history characteristics for fall armyworm also do not fit the qualifications for host races. So it would seem most likely that these two strains are sibling species (Pashley 1986, Pashley 1988).

Questions still exist as to the fall armyworm strain most commonly found in cotton. The stable carbon isotope ratio in adult wings has been used to determine plant host origin. Nagoshi et al. (2007b) found the composition of fall armyworm strains in Brazil closely resembled those found in Texas. Recent studies attempting to evaluate the migratory pattern of fall armyworm suggest that corn-strain individuals in Louisiana, Mississippi, and Alabama are indistinguishable from those sampled in Texas (Nagoshi and Meagher 2008). This similarity would indicate that Texas is the source of populations in these states as fall armyworm populations move in a northeasterly direction. Given the similarities among fall armyworm populations in North and South America, studies in Ecuador have recently indicated that larvae collected in cotton more

closely resemble the C-strain than the R-strain based upon protein polymorphisms. Results of carbon isotope ratio studies indicate that the C-strain is the subpopulation of fall armyworm most common in a cotton environment. The C-strain develops in substantial numbers compared to the R-strain (Nagoshi et al. 2007a). Inter-strain mating can occur in both directions, but variables exist between mating habits. R-strain females prefer to accept C-strain males, resulting in mixed populations, but C-strain females and R-strain males appear to be reproductively incompatible (Whitford 1988, Quisenberry 1991).

Genetic markers and allozyme variants can be used to distinguish the two strains (Nagoshi and Meagher 2004). Differences between strains have a profound effect on crop protection strategies as a result of variation between the two strains in several life history characteristics, including larval development on host plants, mating behaviors, use of food resources, resistance to insecticides, and susceptibility to different plant cultivars including *Bacillus thuringiensis* (Bt) plants (Veenstra 1994, Nagoshi and Meagher 2004).

Fall Armyworm Damage to Crops

Fall armyworm is known to be a generalist pest on a variety of plants, having been reported on over 80 species in 23 families (Pashley 1988). A number of these plant species are crops including corn; sorghum, *Sorghum bicolor* (L.); forage grasses; turf grasses; rice; cotton; and peanut, *Arachis hypogaea* (L.). However, this species shows preferences for grasses such as corn, sorghum, and bermudagrass, which are C₄ plants, as opposed to C₃ plants such as cotton or soybean, *Glycine max* (L.) Merr. (Luginbill 1928, Buntin 1986, Wittwer 1995, McCarty and Miller 2002, Lewter et al. 2006, Nagoshi et al. 2007a).

Fall armyworm can defoliate a variety of plant species, but it appears to prefer C₃ plants, specifically grasses. Once larvae emerge from eggs on foliage, early instars move upwards,

possibly in response to light or gravity. Later instars then move lower in the canopy (Buntin 1986).

Fall armyworm has shown a preference for oviposition on younger corn plants (Buntin 1986). Corn seedlings infested with fall armyworm are capable of being completely defoliated, but can survive as long as the apical meristem is unharmed. Feeding on whorl stage plants generally results in perforated leaves. Feeding injury during the final stages of plant growth usually occurs on the tassel and the ear (reproductive structures). Upon emergence of the tassel, larvae are no longer able to feed in the whorl, and instead move to the developing ear, where damage to silks can reduce pollination, reducing kernel set. Larvae entering through the tip or husk of the ear cause direct kernel damage, while larvae entering at the base of the ear can cause complete ear abortion.

In grain sorghum, fall armyworm larvae feed mainly on leaves, but can also feed directly on the panicle (Buntin 1986). Similarities exist between feeding patterns observed on sorghum and on corn. Young larvae usually feed on expanded leaves and older larvae move to the whorl to feed. During the reproductive stages of plant development, larvae will continue to feed both on leaves and directly on seeds in the panicle.

Fall armyworm also can cause damage to forage grasses and small grains. Forage grasses damaged by fall armyworm include forage sorghum and sudangrass, *S. bicolor*; bermudagrass; Johnsongrass, *Sorghum halopense* (L.); several species of millet; and bahiagrass, *Paspalum notatum* Flueggé (Buntin 1986). In forage grasses, fall armyworm larvae feed on vegetation, with populations increasing during the late summer and early fall. Small grain crops injured by fall armyworm include wheat, *Triticum* spp., rice, and rye, *Secale cereale* (L.). Damage by fall

armyworm to forage crops directly affects the crop yield. The foliage consumed is the harvestable product.

Fall armyworm is considered an occasional pest of cotton (Walton and Luginbill 1916, Luginbill 1928). Fall armyworm eggs have $\approx 89\%$ successful larval eclosion on cotton, while neonate survivorship is only 3%. Instars L1-L5 survived on cotton at rates ranging from 34% to 64% (Ali and Luttrell 1990). Early instars (L1-L3 stages) are found in the lower-to-mid portion of the plant canopy, where they feed on foliage (Cook et al. 2004). The first two instars generally “skeletonize” leaves near the egg mass from which they eclosed. Later instars have the potential to destroy terminals on cotton seedlings (Leigh et al. 1996). Older instars (L4-L6 stages) present mainly within the lower portion of the plant canopy, will feed on fruiting structures (Cook et al. 2004). These older larvae typically injure bracts, large squares and young bolls (capsules). Heavy infestations can injure all fruiting forms (Leigh et al. 1996). Due to the ability of larger larvae to feed internally in fruiting structures, chemical control becomes more difficult, coupled with their increased tolerance to insecticides during later larval instars (Cook et al. 2004). Cotton bolls at any age are susceptible to fall armyworm damage, however, the significant damage on bolls occurs prior to the accumulation of 852 heat units after anthesis (Emfinger et al. 2007). The current literature does not provide a graphic description of fall armyworm injury to individual cotton plant structures, especially on Bt plants.

The majority of feeding on cotton occurs during the last three instars and accounts for $\approx 98\%$ of the foliage or fruit consumed during their life cycle (Sparks 1979). About 85% of this foliage is consumed during the final three to four days of the pest’s life cycle (Robinson 1999).

Pest Status on Cotton

The fall armyworm was listed as the seventh most damaging pest species in the U.S.

cotton belt during 2006 (Williams 2007a), and the fifth most damaging species in 2009 (Williams 2010a). It was reported to have infested approximately 4 million acres in 2006 (Williams 2007a), compared to 1.8 million acres in 2009 (Williams 2010a). In Louisiana, the fall armyworm infested approximately 280,000 acres, ranking it as the fifth most damaging pest species in Louisiana during 2006. This accounts for a total statewide loss of approximately 1600 bales of cotton in that state (Williams 2007a). In 2009, the fall armyworm was reported to have infested over 45,000 acres, with 124 bales of cotton lost due to infestations of this pest (Williams 2010a).

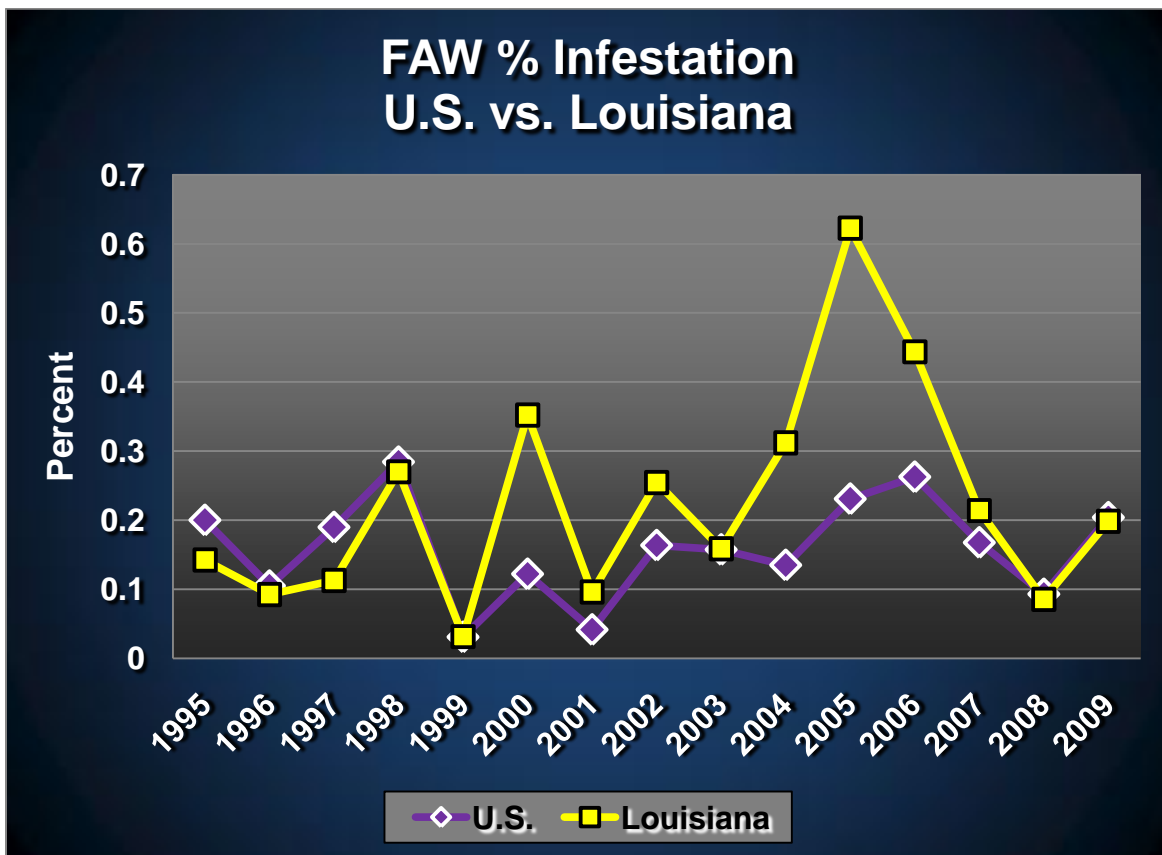


Figure 2.1. Percent annual fall armyworm infestation in U.S. and Louisiana cotton, 1995-2009 (Williams 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007b, and 2008, 2009, 2010b).

Management Strategies

Due to its wide host range and geographical distribution, the fall armyworm can be a very

destructive pest during agricultural cropping seasons (Knipling 1980). Density dependent biological controls are likely negated by the fall armyworm's migratory nature, allowing it to escape many predators, parasitoids, and entomopathogens. A suppression program instituted in common overwintering areas of this species would help to greatly reduce the fall armyworm problem in the southern U.S.

Monitoring for fall armyworm populations can be a helpful tool in assessing populations (Meagher 2001). Colored traps have been evaluated for collecting fall armyworm moths to determine the effectiveness of colors as visual cues for moth capture. The standard trap (green canopy, yellow funnel, white bucket), fluorescent (green canopy, yellow funnel, fluorescent yellow bucket), sun yellow (green canopy, yellow funnel, sun yellow bucket), all-green traps, and all-white traps were evaluated for adult collection efficaciousness. The standard trap (green canopy, yellow funnel, white bucket) was the most effective at accomplishing this goal.

Currently there is little information available about sampling and monitoring fall armyworm infestations in cotton fields. Recommendations for initiating the use of a control strategy are limited to "treat when egg masses or small larvae appear" (Baldwin et al. 2010). The current protocol for sampling fall armyworm in cotton generally follows the recommendations for heliothines (tobacco budworm and bollworm), where cotton fruiting structures are evaluated, and initiation of a control tactic is warranted when a certain % damage or number of larvae are detected. These recommendations do not take into account the behavioral differences between heliothines and fall armyworm, and are thus insufficient for monitoring and controlling fall armyworm infestations.

Cultural Control. Fall armyworm does not possess a diapause mechanism, so cultural control strategies that suppress overwintering populations are ineffective in an annual cropping

system. However, cultural practices do have the ability to influence fall armyworm population during the growing season. Host plant resistance is a key area of concern when attempting to control the fall armyworm. A significant amount of data exists on resistance traits of corn to fall armyworm, but very little data exists on cotton. For corn, antibiosis and non-preference have been the key mechanisms of host plant resistance in successful studies. Fall armyworm-resistant hybrids successfully produce more yield when compared to susceptible hybrids at similar infestation levels (Wiseman et al. 1981, Wiseman et al. 1983, Sparks 1986, Wiseman and Davis 1990).

There are several major obstacles preventing the use of this strategy as an area-wide management technique, including: a majority of the corn crop in overwintering areas is sweet corn, the inability of host plant resistance to withstand heavy infestation, and attempting to convince all growers to use host plant resistance. In most likelihood, host plant resistance will remain a viable pest control tool, but mainly in an on-farm capacity (Sparks 1986). Controlling volunteer plants will also play an important role in preventing fall armyworm populations from building to high numbers. Some fall armyworm resistance to transgenic cotton exists, and allowing volunteer Bt plants to develop provides this species with a habitat that can increase the frequency of resistance in populations.

Biological Control. There are 53 species of parasites, representing 43 genera and 10 families, attacking fall armyworm around the world (Ashley 1979, Sparks 1986). Entomogenous pathogens may be used to suppress insect populations in at least 3 ways: (1) optimization of naturally occurring diseases, (2) introduction and colonization of pathogens into insect populations as natural regulatory agents, and (3) repeated applications of pathogens as microbial insecticides (Gardner and Fuxa 1980).

Several microbial pathogens have been studied in hopes of utilizing them to control fall armyworm populations. Viruses have shown to be effective against fall armyworm, but time-delay required for effects usually allows fall armyworm to cause significant damage prior to insect death (Sparks 1986). Numerous field studies have evaluated entomogenous pathogens to suppress fall armyworm on corn and cabbage, *Brassica oleracea* Capitata Group. Of those tested, *Spodoptera frugiperda* NPV, *Metarrhizium anisopliae* (Metch.) Sorok., and *Neoaplectana carpocapsae* Weiser showed effective control of fall armyworm. Foliar sprays of Bt showed effective control of fall armyworm on cabbage, but with mixed results depending upon formulation (Gardner and Fuxa 1980). Those results suggested rates too high to be economically feasible. FAW-specific Bt isolates have not been developed for commercial spray formulations (Sparks 1986). However, the Cry1F Bt protein is generally considered to be more toxic to fall armyworm than other Cry proteins (Tindall et al. 2006, Adamczyk et al. 2008).

Three common species of fall armyworm parasitoids prevalent to the southeastern U.S. are *Chenolus insularis* Cresson, *Temelucha difficilis* Dasch, and *Cotesia marginiventris* (Cresson). In South Florida, attempts to release two fall armyworm parasitoids (*Eiphosoma vitticole* Cresson and *Telenomus remus* Nixon) were unsuccessful. Recent attempts to mass-rear larval parasitoids of fall armyworm have been unsuccessful (Gross and Pair 1986, Sparks 1986). Lewis and Nordlund (1980) proposed three possible mass rearing and release approaches to controlling the fall armyworm: (1) release throughout the overwintering zone, (2) early-season colonization, and (3) direct therapeutic release on target crops. Of these approaches, two likely successful organisms, *Apanteles marginiventris* (Cresson) and *Chelonus insularis* (Cresson) (Lewis and Nordlund 1980) have been proposed as candidates.

In additional studies, *Archytas marmoratus* (Townsend) has been successfully mass-reared and released (Sparks 1986). It is a parasitoid of noctuids, including fall armyworm, so possibilities exist for successfully utilizing this parasite in fall armyworm overwintering areas to suppress populations.

Further interest in controlling fall armyworm through biological methods has led to the pursuit of semiochemicals which would alter the behavior of fall armyworm in order to suppress population development (Sparks 1986). While entomologists conducting this research are optimistic, there has yet to be any demonstration of its usefulness.

The current literature does not explicitly describe predators which attack only fall armyworm. Many predators are known to attack this species, but there is no summary information available which describes these predators only for fall armyworm (Lewis and Nordlund 1980, Sparks 1986). While predators have an effect on fall armyworm survival and development, their role is largely undermined by parasitoids, which are more efficient in affecting fall armyworm populations. This is due to the difference between a predator's food consumption limitations and a parasitoid's reproductive limitations. A predator is limited in consumption by the amount of food it can consume before progressing to its next life stage, while a parasitoid has the ability to reproduce as long as it lives.

Conventional Chemical Control. Fall armyworm control usually has been accomplished incidentally, with insecticide applications used to control the bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.), collectively known as the heliothine complex. Insecticides have long been prominent in controlling more common pests, such as heliothines, but the majority of these compounds have been largely ineffective in controlling the fall armyworm. Fall armyworm larvae generally are not discovered

until they are late instars, and grower preference leans toward eliminating the problem with a single insecticide application. However, once a fall armyworm population has been established, two applications are often needed for successful control (Sullivan et al. 1999). The dispersion of fall armyworm larvae lower in the plant canopy makes them more difficult to control. This problem is due to the difficulty in insecticide applications penetrating the plant canopy to the location of larvae (Ali et al. 1990, Adamczyk et al. 1997, Adamczyk et al. 1999, Cook et al. 2004). In addition, larvae become increasingly tolerant to insecticides with increased age / size (Yu 1983, Mink and Luttrell 1989). Since the inception of Bt cotton, incidental control of this species has further declined. Not only are fewer insecticide applications used to control Lepidopteran pests, but the majority of insecticides used today are target-specific, further reducing incidental fall armyworm control when treating for other pests.

Fall armyworm susceptibility to insecticides also appears dependent upon larval host plants (Wood 1979). The majority of studies involving chemical control of fall armyworm have been laboratory studies, with little evaluation of the insecticidal effects on fall armyworm in the field.

Successful chemical control strategies usually necessitate the use of full rates of these insecticides (Adamczyk and Sumerford 2000). Fall armyworm has developed resistance to several classes of insecticides, including pyrethroids (cypermethrin, fenvalerate, fluvalinate, permethrin), organophosphates (chlorpyrifos, methyl parathion, diazinon, malathion, and trichlorfon), and carbamates (methomyl, carbaryl, and thiodicarb) (Wood et al. 1981, Yu 1992, Adamczyk et al. 1999, Al-Sarar et al. 2006, Whalon et al. 2008). Pyrethroids (permethrin, cypermethrin, flucythrinate, etc.) studied in the 1980's showed a range of activity as fall armyworm oviposition repellents at ovicidal concentrations, with levels of repellency ranging

from 96% to 50% depending on dose. At 10% of ovicidal concentrations, the range varied from 90% to 32% (Gist and Pless 1985). In studies of the ovicidal activity of permethrin (Ambush), a higher percentage of fall armyworm eggs hatched when exposed to field rates compared to corn earworm and cabbage looper (Tysowsky and Gallo 1977). Insecticides such as carbaryl, trichlorfon, methyl parathion, pyrethroids and ethyl parathion provide little to no control of fall armyworm, compared to effective control with chlorpyrifos, sulprofos, thiodicarb, and methomyl (Pitre 1986).

The toxicity of insecticide residue on excised leaves and white flowers to fall armyworm (first instar, one-day-old larvae) was evaluated for several insecticides, including chlorfenapyr, emamectin benzoate, lambda-cyhalothrin, methoxyfenozide, spinosad, and thiodicarb (Adamczyk et al. 1999). Chlorfenapyr, lambda-cyhalothrin, and thiodicarb residue on leaves resulted in significantly higher mortality than the non-treated control at 24 and 48 hours after infestation (HAI), while emamectin benzoate and spinosad resulted in significantly higher mortality only at 48 HAI. At 24 and 48 HAI, no insecticide resulted in greater than 70% and 88% mortality, respectively. On white flowers, chlorfenapyr, emamectin benzoate, lambda-cyhalothrin, spinosad, and thiodicarb resulted in significantly higher mortality than the non-treated control at 24 and 48 HAI with methoxyfenozide resulting in significantly higher mortality only at 48 HAI. At 24 and 48 HAI, no insecticide resulted in greater than 74% and 95% mortality, respectively. Chlorfenapyr, emamectin benzoate, lambda-cyhalothrin, and thiodicarb on cotton bolls resulted in significantly higher mortality than the non-treated control at 3, 5, and 7 days after infestation (DAI), while methoxyfenozide only resulted in significantly higher mortality at 7 DAI. At 3, 5, and 7 DAI, no insecticide resulted in greater than 43%, 53%, and 58% mortality, respectively.

In recent studies, novaluron has been shown to provide effective control of Southern armyworm, *Spodoptera eridania*, (Angle and Weiland 2006) but has not been evaluated in detail on subsequent *Spodoptera* species. Field treatments of methoxyfenozide in 2001 reduced fall armyworm infestation and boll damage on Bt cotton (Walton et al. 2001). In adult vial tests, fall armyworm appeared to be less susceptible to indoxacarb in comparison to bollworm and tobacco budworm. However, fall armyworm appeared to be equally or more susceptible to indoxacarb in meridic diet overlay assays (Cook et al. 2001). Control of fall armyworm with available insecticides is still not well defined. Research is needed to determine effective control rates with new insecticides, such as chlorantraniliprole and flubendiamide, specific to fall armyworm. In diet-incorporated assays, fall armyworm appears to be equally susceptible to chlorantraniliprole compared to bollworm and tobacco budworm (Temple et al. 2009).

Currently, the recommended insecticides to control fall armyworm include pyrethroids (lambda-cyhalothrin, zeta-cypermethrin, etc.), carbamates (thiodicarb), spinosyns (spinosad), and insect growth regulators (benzoylureas [novaluron] and diacylhydrazines [methoxyfenozide]) (Baldwin et al. 2010, IRAC Mode of Action Working Group 2010). The rates of recommended products to control fall armyworm are equal to or above the recommended rates to control bollworm and tobacco budworm (Baldwin et al. 2010). However, based upon available information in the Arthropod Management Tests (Entomological Society of America, www.entsoc.org), limited insecticide efficacy data for control of fall armyworm exists, particularly from field experiments. In addition, there are newly registered insecticides on cotton for which no data on fall armyworm control exists.

Bt Traits in Transgenic Cotton. The introduction of Bt cotton in 1996 containing the Cry1Ac endotoxin exhibited effective control of lepidopteran pests such as the tobacco budworm

and pink bollworm (Adamczyk and Gore 2004). The Cry1Ac endotoxin has shown less effective control of bollworm, fall armyworm, beet armyworm, and soybean looper (Stewart et al. 2000). Fall armyworm has the ability to damage Bollgard[®] bolls to a more extensive degree than other pests such as the bollworm, tobacco budworm, and beet armyworm (Adamczyk and Sumerford 2000). The bollworm is considerably more tolerant to Cry1Ac than the tobacco budworm, with the fall armyworm exhibiting an even higher tolerance (Stewart et al. 2000). In 2008, LC₅₀ values were >100 µg/ml for Cry1Ac and 82 µg/ml for Cry2Ab for fall armyworm neonates (Sivasupramaniam et al. 2008).

Studies have also shown a difference in fall armyworm preference between cotton expressing Cry proteins (Bt cotton) and those that do not express proteins (non-Bt cotton) (Akin et al. 2001a). Fall armyworm larvae placed on tissue incorporated diet did not show a significant preference for any of three lines (non-Bt, Bollgard[®], and Bollgard II[®]) 6 HAI. After six hours, significantly more larvae had migrated from non-Bt and Bollgard II[®] treatments than from the Bollgard[®] treatment. At 24 HAI, significantly fewer larvae were found on Bollgard II[®] than on either Bollgard[®] or non-Bt. After 24 hours, more larvae had moved from the Bollgard II[®] treatment than from the Bollgard[®] or non-Bt treatments. After six hours, neonates were almost twice as likely to have moved from the treatment on which they were placed than second instars. After 24 hours, second instars moved from the treatment on which they were placed significantly more than neonates. These results suggest a correlation between the ingestion of Bt toxins and larval movement. However, Chitkowski et al. (2003) compared non-Bt, Bollgard[®], and Bollgard II[®] varieties in field trials and found no significant differences in number of larvae recorded among varieties. These results indicate discrepancies in available data for fall armyworm

behavior on transgenic cotton. Fall armyworm preference, or non-preference, for Bt or non-Bt cotton remains unclear at this time due to conflicting data.

The widespread use of available Bt technologies (>90% in Louisiana) necessitates extensive research on the current and future status of such a prominent insect control tool. Cotton acreage in Louisiana, and eventually the U.S., will be saturated with the maximum allowable Bt acreage (95%). As we near that time, there is still much to learn about the effects of Bt cotton on a sporadic, yet potentially devastating pest such as the fall armyworm.

Efficacy of Bollgard[®]. The Cry1Ac endotoxin in Bollgard[®] cotton has demonstrated little efficacy against fall armyworm in field and laboratory experiments (Adamczyk et al. 1998, Adamczyk and Gore 2004). Larval weights (previous generation reared on conventional cotton leaves, Bt cotton leaves, and non-Cry1Ac meridic diet) were significantly lower on diet containing Cry1Ac tissue compared to non-Cry1Ac diet (Adamczyk and Sumerford 2000). Time to pupation (previous generation reared on conventional cotton leaves, Bt cotton leaves, and non-Cry1Ac diet) was significantly longer on Cry1Ac diet compared to non-Cry1Ac diet. Survival to pupation also was significantly reduced on Cry1Ac diet compared to non-Cry1Ac diet, but only in colonies exhibiting slow and regular development. Larval weights (fast, slow and regular developmental times) were significantly reduced on Cry1Ac diet compared to non-Cry1Ac diet. However, mean time to pupation was significantly increased on Cry1Ac diet compared to non-Cry1Ac diet. Cry1Ac produces sub-lethal effects on fall armyworm within a defined time period. In laboratory assays, Adamczyk et al. (2008) observed no differences in survival between neonates fed mid-canopy leaves from Bollgard[®] plants and non-Bt plants. Similar results were recorded when larvae were offered leaves from lower in the canopy.

Studies in Louisiana have shown that significantly fewer fall armyworm larvae were observed in Bollgard[®] plots compared to that in non-Bt plots. Fruiting form abscission, damaged bracts, and penetrated bolls were not significantly different between Bollgard[®] and non-Bt plots (Leonard et al. 2006). In Mississippi studies, Bollgard[®] cotton did not significantly differ from the non-Bt plots in numbers of larvae in white flowers, pink flowers, or on whole plants. Bollgard[®] cotton had significantly fewer damaged bolls, as well as significantly higher yield, than that found in conventional non-Bt plots. Bollgard[®] plots did not significantly differ from the non-Bt plots in numbers of damaged squares or pink flowers (Adamczyk et al. 2001).

Efficacy of Bollgard II[®]. Bollgard II[®] plants produce Cry1Ac and Cry2Ab endotoxins. In laboratory studies, mortality of second-instar fall armyworms was significantly higher on leaves, squares and bolls of Bollgard II[®] cotton compared to larvae exposed to the same structures of Bollgard[®] and non-Bt cotton (Chitkowski et al. 2003). In field experiments, Bollgard II[®] did not significantly improve control of fall armyworm above Bollgard[®] (Leonard et al. 2006). Bollgard II[®] had significantly fewer fall armyworm larvae than the non-Bt plots. In laboratory experiments, Bollgard II[®] outperformed Bollgard[®] against fall armyworm, but remained susceptible to damage by later larval instars (Leonard et al. 2006).

Fall armyworm larvae (second instars) were evaluated in bioassays to determine the effects of Cry1Ac alone and Cry1Ac/Cry2Ab in comparison with a non-Bt control. Larvae were infested on terminal leaf discs collected from plants at flowering, 2 weeks after flowering (WAF), and 4 WAF. Both Cry1Ac and Cry1Ac/Cry2Ab resulted in significantly higher mortality and lower larval weights than the non-Bt control at flowering. At 2 and 4 WAF, Cry1Ac/Cry2Ab resulted in significantly higher mortality and lower average larval weight than Cry1Ac alone and the non-Bt control (Sivasupramaniam et al. 2008).

Adamczyk et al. (2001) found that Bollgard II[®] did not significantly reduce fall armyworm (numbers) found in white flowers below that in Bollgard[®] or non-Bt cotton. Bollgard II[®] did significantly reduce fall armyworm larvae in pink flowers and on whole plants compared to that on non-Bt cotton. Bollgard II[®] cotton resulted in significantly fewer damaged pink flowers and bolls than that on non-Bt cotton, but did not significantly affect numbers of damaged squares (Adamczyk et al. 2001). Bollgard II[®] resulted in significantly less feeding by larvae (second instars) compared to Bollgard[®] and non-Bt treatments. In addition, the proportion of surviving larvae was also affected, with significantly fewer survivors on Bollgard II[®] after 3 days compared to those on Bollgard[®] and non-Bt plants. For the remaining evaluation dates, Bollgard II[®] plants significantly reduced survivors compared to Bollgard[®]. The length of larvae (recorded in mm) at 5 and 7 DAI was significantly less for larvae exposed to Bollgard II[®] compared to those exposed to Bollgard[®] and non-Bt treatments. Bollgard II[®] also significantly delayed pupation compared to Bollgard[®] and non-Bt treatments (Stewart et al. 2001). In Mississippi field trials, significantly fewer fall armyworm larvae were found in Bollgard II[®] cotton than were found in Bollgard[®] or non-Bt cotton (Akin et al. 2001). Adamczyk et al. (2008) observed significantly higher mortality for neonates fed mid and lower canopy leaves from Bollgard II[®] plants compared to those offered leaves from non-Bt plants. Mortality was also greater on Bollgard II[®] tissue compared to Bollgard[®] tissue.

Given the amount of work with fall armyworm on Bollgard II[®] cotton, questions still remain. Should a serious fall armyworm outbreak occur, it is still unknown if this species can significantly injure Bollgard II[®]. Available data suggests favorable control of early instars, yet control becomes an issue as larvae age. Should fall armyworm larvae progress to late instars on

adjacent plants (non-Bt cotton, weeds), insufficient information is available to deny the need for supplemental control strategies in Bollgard II[®].

Efficacy of WideStrike[™]. WideStrike[™] cotton produces the Cry1Ac endotoxin found in Bollgard[®] and Bollgard II[®] cotton varieties. WideStrike[™] also contains a second protein, Cry1F, which provides control of selected *Spodoptera* spp. In laboratory assays, comparing conventional non-Bt, Cry1Ac, Cry1F, and Cry1Ac + Cry1F (WideStrike[™]) cotton lines, the Cry1F alone and WideStrike[™] lines resulted in significantly lower survivorship on cotton squares compared to that on Cry1Ac and non-Bt squares. Neonates fed WideStrike[™] squares and leaves had significantly lower survival compared to those on non-Bt forms (Adamczyk and Gore 2004). Adamczyk et al. (2008) reported neonate mortality on WideStrike[™] leaves (both mid and lower canopy) to be greater than that on non-Bt and Bollgard[®] leaves. Tindall et al. (2009) observed a significant decrease in second-instar survivorship and a decrease in plant damage when larvae were offered leaves and squares from WideStrike[™] plants compared to those from non-Bt plants. In field trials WideStrike[™] exhibited significantly fewer abscised bolls than non-Bt cotton, but no significant differences were observed for % damaged bracts, % penetrated bolls, or number of fall armyworm larvae. Siebert et al. (2008) reported fewer fall armyworm larvae in non-Bt plots compared to that in WideStrike[™] plots under natural infestation. In additional field experiments, fewer WideStrike[™] fruiting forms (squares, flowers, and bolls) were infested with fall armyworm larvae compared to non-Bt fruiting forms. Expression of Cry1Ac and Cry1F was evaluated by Siebert et al. (2009) who found that protein expression varied significantly both among plant tissues and throughout the season. For effects on fall armyworm, only a limited amount of efficacy data exists with WideStrike[™]. It will become increasingly important to closely examine the effects of this technology on fall armyworm, as there are already reports of fall armyworm

surviving on Cry1F corn in Puerto Rico. Fall armyworm resistance to Cry1F, coupled with its limited susceptibility to Cry1Ac, points toward a potentially serious management issue in the future.

Efficacy of VipCot™. VipCot™ cotton contains the novel vegetative insecticidal protein, Vip3A. VipCot™ varieties in development express the Vip3A protein stacked with the Bt endotoxin, Cry1Ab (Adamczyk and Mahaffey 2007). VipCot™ cotton lines produced significantly higher fall armyworm larval mortality than on non-Bt and Vip3A lines (Adamczyk and Mahaffey 2007). VipCot™ efficacy data on fall armyworm is extremely limited at the present time, with no available data on commercial lines. Fundamental data on fall armyworm survival and behavior on commercial VipCot™ plants must be collected to determine the ability of this technology to withstand fall armyworm infestations.

Efficacy of Bayer Transgenic Lines. Transgenic Bt varieties, commercially labeled as TwinLink, are currently under development by Bayer CropScience. This technology is not currently available. The Louisiana State University AgCenter is one of a select group of universities cooperating with Bayer CropScience to evaluate the development of their proprietary single and dual proteins in cotton. These Cry proteins are being evaluated under secrecy agreements with the LSU AgCenter. No data has been published on these developmental lines, and intrinsic research involving its effects will become important to characterize the effects of this technology against fall armyworm.

References Cited

Adamczyk, Jr., J. J., J. W. Holloway, B. R. Leonard, and J. B. Graves. 1997. Defining the period of boll susceptibility to fall armyworm injury in cotton, pp. 941-944. *In Proc.* 1997 Beltwide Cotton Conf., New Orleans, LA. January 6-10, 1997.

Adamczyk, Jr., J. J., J. W. Holloway, G. E. Church, B. R. Leonard, and J. B. Graves. 1998. Larval survival and development of the fall armyworm (Lepidoptera: Noctuidae) on

- normal and transgenic cotton expressing the *Bacillus thuringiensis* CryIA(c) δ -endotoxin. *J. Econ. Entomol.* 91: 539-545.
- Adameczyk, Jr., J. J., B. R. Leonard, and J. B. Graves. 1999.** Toxicity of selected insecticides to fall armyworms (Lepidoptera: Noctuidae) in laboratory bioassay studies. *Florida Entomol.* 82: 231-236.
- Adameczyk, Jr., J. J. and D. V. Sumerford. 2000.** Increased tolerance of fall armyworms (Lepidoptera: Noctuidae) to Cry1Ac δ -endotoxin when fed transgenic *Bacillus thuringiensis* cotton: Impact on the development of subsequent generations. *Florida Entomol.* 84: 1-6.
- Adameczyk, 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: 1589-1593.
- Adameczyk, Jr., J. J. and J. Gore. 2004.** Laboratory and field performance of cotton containing Cry1Ac, Cry1F, and both Cry1Ac and Cry1F (WideStrike®) against beet armyworm and fall armyworm larvae (Lepidoptera: Noctuidae). *Florida Entomol.* 87: 427-432.
- Adameczyk, Jr., J. J. and J. S. Mahaffey. 2007.** Efficacy of Vip3A and Cry1Ab genotypes against various lepidopteran pests, pp. 1106-1113. *In Proc. 2007 Beltwide Cotton Conf., New Orleans, LA. January 9-12, 2007.*
- Adameczyk, J. J., Jr., S. Greenberg, J. S. Armstrong, W. J. Mullins, L. B. Braxton, R. B. Lassiter, and M. W. Siebert. 2008.** Evaluations of Bollgard®, Bollgard II®, and WideStrike® technologies against beet and fall armyworm larvae (Lepidoptera: Noctuidae). *Florida Entomol.* 91: 531-536.
- Akin, D. S., S. D. Stewart, and K. S. Knighten. 2001a.** Behavioral response of Lepidopteran pests on cotton expressing insecticidal proteins of *Bacillus thuringiensis*, pp. 828-830. *In Proc. 2001 Beltwide Cotton Conf., Anaheim, CA. January 9-13, 2001.*
- Akin, D. S., S. D. Stewart, and K. S. Knighten. 2001b.** Field efficacy of cotton expressing two insecticidal proteins of *Bacillus thuringiensis*, pp. 1041-1043. *In Proc. 2001 Beltwide Cotton Conf., Anaheim, CA. January 9-13, 2001.*
- Al-Sarar, A., F. R. Hall, and R. A. Downer. 2006.** Impact of spray application methodology on the development of resistance to cypermethrin and spinosad by fall armyworm *Spodoptera frugiperda* (J.E. Smith). *Pest Management Sci.* 62: 1023-1031.
- Ali, A., R. G. Luttrell, H. N. Pitre, and F. M. Davis. 1989.** Distribution of fall armyworm (Lepidoptera: Noctuidae) egg masses on cotton. *Environ. Entomol.* 18: 881-885.

- Ali, A., R. G. Luttrell, and H. N. Pitre. 1990.** Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. *Environ. Entomol.* 19: 1060-1067.
- Ali, A. and R. G. Luttrell. 1990.** Survival of fall armyworm (Lepidoptera: Noctuidae) immature on cotton. *Florida Entomol.* 73: 459-465.
- Angle, Jr., G. W. and R. T. Weiland. 2006.** Diamond insecticide: broad spectrum control of heliothines, loopers, and armyworms in addition to lygus, stinkbugs, and fleahoppers. *In Proc. 2006 Beltwide Cotton Conf., San Antonio, TX. January 3-6, 2006.*
- Ashley, T. R. 1979.** Classification and distribution of fall armyworm parasites. *Florida Entomol.* 62: 114-122.
- Ashley, T. R., B. R. Wiseman, F. M. Davis, and K. L. Andrews. 1989.** The fall armyworm: a bibliography. *Florida Entomol.* 72: 152-202.
- Baldwin, J. L., L. D. Foil, M. L. Grodner, A. Hammond, G. Henderson, N. Hummel, S. Johnson, R. Leonard, A. Morgan, D. K. Pollet, J. Pyzner, T. E. Reagan, D. Reed, D. Ring, R. N. Story, and M. Stout. 2010.** 2010 Louisiana Insect Pest Management Guide. LSU AgCenter [Online]. Available at http://www.lsuagcenter.com/en/communications/publications/management_guides/Louisiana+Insect+Pest+Management+Guide.htm (verified 16 May 2011).
- Buntin, G. D. 1986.** A review of plant response to fall armyworm, *Spodoptera frugiperda* (J.E. Smith), injury in selected field and forage crops. *Florida Entomol.* 69: 549-559.
- Chitkowski, R. L., S. G. Turnipseed, M. J. Sullivan, and W. C. Bridges, Jr. 2003.** Field and laboratory evaluations of transgenic cottons expressing one or two *Bacillus thuringiensis* var. *kurstaki* Berliner proteins for management of Noctuid (Lepidoptera) pests. *J. Econ. Entomol.* 96: 755-762.
- Cook, D. R., B. R. Leonard, and J. Gore. 2001.** Evaluation of spinosad, indoxacarb, and S-1812 against selected Lepidopteran pests, pp. 808-811. *In Proc. 2001 Beltwide Cotton Conf., Anaheim, CA. January 9-13, 2001.*
- Cook, D. R., B. R. Leonard, and J. Gore. 2004.** Field and laboratory performance of novel insecticides against armyworms (Lepidoptera: Noctuidae). *Florida Entomol.* 87: 433-439.
- Dew, J. A. 1913.** Fall army worm. *J. Econ. Entomol.* 6: 361-366.
- Emfinger, K. D., B. R. Leonard, and P. L. Bommireddy. 2007.** Validating boll and yield tolerance to cotton pests in Louisiana, pp. 118-122. *In Proc. 2007 Beltwide Cotton Conf., New Orleans, LA. January 9-12, 2007.*

- Foster, R. E. and R. H. Cherry. 1987.** Survival of fall armyworm, *Spodoptera frugiperda*, (Lepidoptera: Noctuidae) exposed to cold temperatures. Florida Entomol. 70: 419-422.
- Gardner, W. A. and J. R. Fuxa. 1980.** Pathogens for the suppression of the fall armyworm. Florida Entomol. 63: 439-447.
- Gist, G. L. and C. D. Pless. 1985.** Ovicidal activity and ovipositional repellent properties of synthetic pyrethroids to the fall armyworm, *Spodoptera frugiperda*. Florida Entomol. 68: 462-466.
- Gross, H. R., Jr. and S. D. Pair. 1986.** The fall armyworm: status and expectations of biological control with parasitoids and predators. Florida Entomol. 69: 502-515.
- IRAC International MoA Working Group. 2010.** IRAC MoA classification scheme. Version 7.0 [Online]. Available at http://www.iraconline.org/wp-content/uploads/2009/09/MoA-classification_v7.0.4-5Oct10.pdf (verified Mar. 28, 2011).
- Jackson, R., J. Gore, and A. Catchot. 2007.** Impact of fall armyworm survival in Bt crops on survival and damage potential of subsequent generations, pp. 1634-1637. *In Proc. 2007 Beltwide Cotton Conf., New Orleans, LA. January 9-12, 2007.*
- Knipling, E. F. 1980.** Regional management of the fall armyworm—a realistic approach? Florida Entomol. 63: 468-480.
- Leigh, T. F., S. H. Roach, and T. F. Watson. 1996.** Biology and ecology of important insect and mite pests of cotton, pp. 17-85. *In E. G. King, J. R. Phillips, and R. J. Coleman (eds.) Cotton insects and mites: characterization and management.* (No. 3) The Cotton Foundation Reference Book Series.
- Leonard, B. R., K. V. Tindall, and K. D. Emfinger. 2006.** Fall armyworm survivorship and damage in Bollgard and Bollgard 2 cotton, pp. 1080-1084. *In Proc. 2006 Beltwide Cotton Conf., San Antonio, TX. January 3-6, 2006.*
- Levy, H. C., A. Garcia-Maruniak, and J. E. Maruniak. 2002.** Strain identification of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) insects and cell line: PCR-RFLP of cytochrome oxidase C subunit I gene. Florida Entomol. 85: 186-190.
- Lewis, W. J. and D. A. Nordlund. 1980.** Employment of parasitoids and predators for fall armyworm control. Florida Entomol. 63: 433-438.
- Lewter, J. A., A. L. Szalanski, R. N. Nagoshi, R. L. Meagher, Jr., C. B. Owens, and R. G. Luttrell. 2006.** Genetic variation within and between strains of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Florida Entomol. 89: 63-68.
- Luginbill, P. 1928.** The fall armyworm. USDA Tech. Bull. No. 34.

- Luttrell, R. G. and J. S. Mink. 1999.** Damage to cotton fruiting structures by the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *J. Cotton Science* 3: 35-44.
- McCarty, L. B. and G. Miller. 2002.** Managing Bermudagrass Turf: Selection, Construction, Cultural Practices, and Pest Management Strategies. John Wiley and Sons. 221 pp.
- Meagher, R. L., Jr. 2001.** Collection of fall armyworm (Lepidoptera: Noctuidae) adults and nontarget Hymenoptera in different colored unitraps. *Florida Entomol.* 84: 77-82.
- Mink, J. S. and R. G. Luttrell. 1989.** Mortality of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs, larvae and adults exposed to several insecticides on cotton. *J. Entomol. Sci.* 24: 563-571.
- Nagoshi, R. N. and R. L. Meagher. 2004.** Seasonal distribution of fall armyworm (Lepidoptera: Noctuidae) host strains in agricultural and turf grass habitats. *Environ. Entomol.* 33: 881-889.
- Nagoshi, R. N. and R. L. Meagher. 2008.** Review of fall armyworm (Lepidoptera: Noctuidae) genetic complexity and migration. *Florida Entomol.* 91: 546-554.
- Nagoshi, R. N., J. J. Adamczyk, Jr., R. L. Meagher, J. Gore, and R. Jackson. 2007a.** Using stable isotope analysis to examine fall armyworm (Lepidoptera: Noctuidae) host strains in a cotton habitat. *J. Econ. Entomol.* 100: 1569-1576.
- Nagoshi, R. N., P. Silvie, R. L. Meagher, J. Lopez, and V. Machado. 2007b.** Identification and comparison of fall armyworm (Lepidoptera: Noctuidae) host strains in Brazil, Texas, and Florida. *Ann. Entomol. Soc. Am.* 100: 394-402.
- Oliver, A. D. and J. B. Chapin. 1981.** Biology and illustrated key for the identification of twenty species of economically important noctuid pests. *Louisiana Agric. Expt. Stn. Bull. No.* 733.
- Pair, S. D., J. R. Raulston, A. N. Sparks, J. K. Westbrook, and G. K. Douce. 1986.** Fall armyworm distribution and population dynamics in the southeastern states. *Florida Entomol.* 69: 468-487.
- Pashley, D. P. 1986.** Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex. *Ann. Entomol. Soc. Am.* 79: 898-904.
- Pashley, D. P. 1988.** Current status of fall armyworm host strains. *Florida Entomol.* 71: 227-234.
- Pashley, D. P., T. N. Hardy, and A. M. Hammond. 1995.** Host effects on developmental and reproductive traits in fall armyworm strains (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 88: 748-755.

- Pitre, H. N. 1986.** Chemical control of the fall armyworm (Lepidoptera: Noctuidae): an update. Florida Entomol. 69: 570-578.
- Quisenberry, S. S. 1991.** Fall armyworm (Lepidoptera: Noctuidae) host strain reproductive compatibility. Florida Entomol. 74: 194-199.
- Robinson, J. V. 1999.** Biology of the fall armyworm. Accessed July 5, 2007 [On-line] <http://overton2.tamu.edu/insects/fawbio.pdf>.
- Siebert, M. W., J. M. Babock, S. Nolting, A. C. Santos, J. J. Adamczyk, Jr., P. A. Neese, J. E. King, J. N. Jenkins, J. McCarty, G. M. Lorenz, D. D. Fromme, and R. B. Lassiter. 2008.** Efficacy of Cry1F insecticidal protein in maize and cotton for control of fall armyworm (Lepidoptera: Noctuidae). Florida Entomol. 91: 555-565.
- Siebert, M. W., T. G. Patterson, G. J. Gilles, S. P. Nolting, L. B. Braxton, B. R. Leonard, J. W. Van Duyn, and R. B. Lassiter. 2009.** Quantification of Cry1Ac and Cry1F *Bacillus thuringiensis* insecticidal proteins in selected transgenic cotton plant tissue types. J. Econ. Entomol. 102: 1301-1308.
- Simmons, A. M. 1993.** Effects of constant and fluctuating temperatures and humidities on the survival of *Spodoptera frugiperda* pupae (Lepidoptera: Noctuidae). Florida Entomol. 76: 333-340.
- Sivasupramaniam, S., W. J. Moar, L. G. Ruschke, J. A. Osborn, C. Jiang, J. L. Sebaugh, G. R. Brown, Z. W. Shappley, M. E. Oppenhuizen, J. W. Mullins, and J. T. Greenplate. 2008.** Toxicity and characterization of cotton expressing *Bacillus thuringiensis* Cry1Ac and Cry2Ab2 proteins for control of Lepidopteran pests. J. Econ. Entomol. 101: 546-554.
- Sparks, A. N. 1979.** A review of the biology of the fall armyworm. Florida Entomol. 62: 82-87.
- Sparks, A. N. 1986.** Fall armyworm (Lepidoptera: Noctuidae): potential for area-wide management. Florida Entomol. 69: 603-614.
- Stewart, S. D., K. S. Knighten, and F. M. Davis. 2000.** Efficacy of Bt cotton expressing two insecticidal proteins of *Bacillus thuringiensis* Berliner on selected caterpillar pests, pp. 1043-1049. In Proc. 2000 Beltwide Cotton Conf., San Antonio, TX. January 4-8, 2000.
- 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: 752-760.
- Sullivan, M. J., S. G. Turnipseed, and D. Robinson. 1999.** Insecticidal efficacy against a complex of fall and beet armyworms and soybean looper in South Carolina cotton, pp. 1034-1036. In Proc. 1999 Beltwide Cotton Conf., Orlando, FL. January 3-7, 1999.

- Temple, J. H., P. L. Bommireddy, D. R. Cook, P. Marçon, and B. R. Leonard. 2009.** Susceptibility of selected Lepidopteran pests to Rynaxypyr®, a novel insecticide. *J. Cotton Sci.* 13: 23-31.
- Tindall, K. V., B. R. Leonard, and K. D. Emfinger. 2006.** Fall armyworm survivorship and damage in WideStrike cotton, pp. 1541-1544. *In Proc. 2006 Beltwide Cotton Conf., San Antonio, TX. January 3-6, 2006.*
- Tindall, K. V., M. W. Siebert, B. R. Leonard, J. All, and F. J. Haile. 2009.** Efficacy of Cry1Ac:Cry1F proteins in cotton leaf tissue against fall armyworm, beet armyworm, and soybean looper (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 102: 1497-1505.
- Tysowsky, M. and T. Gallo. 1977.** Ovicidal activity of Ambush™, a synthetic pyrethroid insecticide, on corn earworm, fall armyworm, and cabbage looper. *Florida Entomol.* 60: 287-290.
- Veenstra, K. H. 1994.** Host plant adaptation in the two strains of fall armyworm (Lepidoptera: Noctuidae). Dissertation: Dept. of Entomology, Louisiana State University and Agricultural and Mechanical College. 107 pp.
- Walton, L. C., J. W. McGee, and J. A. Spivey. 2001.** Performance of Intrepid 80WSP under section 18 exemption in mid-south cotton, pp. 993-996. *In Proc. 2001 Beltwide Cotton Conf., Anaheim, CA. January 9-13, 2001.*
- Walton, W. R. and P. Luginbill. 1916.** The fall armyworm, or grassworm, and its control. *USDA Farmer's Bull. No. 752.*
- Whalon, M. E., D. Mota-Sanchez, R. M. Hollingworth, and L. Duynslager. 2008.** Arthropod pesticide resistance database. Accessed Sept. 25, 2008 [Online] <http://www.pesticideresistance.org/search/12/0/200/0/>.
- Whitford, F., S. S. Quisenberry, T. J. Riley, and J. W. Lee. 1988.** Oviposition preference, mating compatibility, and development of two fall armyworm strains. *Florida Entomol.* 71: 234-243.
- Williams, M. R. 1996.** Cotton insect losses 1995, pp. 670-689. *In Proc. 1996 Beltwide Cotton Conf., Nashville, TN. January 9-12, 1996.*
- Williams, M. R. 1997.** Cotton insect losses 1996, pp. 834-853. *In Proc. 1997 Beltwide Cotton Conf., New Orleans, LA. January 6-10, 1997.*
- Williams, M. R. 1998.** Cotton insect losses 1997, pp. 904-925. *In Proc. 1998 Beltwide Cotton Conf., San Diego, CA. January 5-9, 1998.*

- Williams, M. R. 1999.** Cotton insect losses 1998, pp. 785-808. *In Proc. 1999 Beltwide Cotton Conf.*, Orlando, FL. January 3-7, 1999.
- Williams, M. R. 2000.** Cotton insect losses 1999, pp. 887-913. *In Proc. 2000 Beltwide Cotton Conf.*, San Antonio, TX. January 4-8, 2000.
- Williams, M. R. 2001.** Cotton insect losses 2000. *In Proc. 2001 Beltwide Cotton Conf.*, Anaheim, CA. January 9-13, 2001.
- Williams, M. R. 2002.** Cotton insect losses 2001. *In Proc. 2002 Beltwide Cotton Conf.*, Atlanta, GA. January 8-12, 2002.
- Williams, M. R. 2003.** Cotton insect loss estimates - 2002, pp. 1217-1273. *In Proc. 2003 Beltwide Cotton Conf.*, Nashville, TN. January 6-10, 2003.
- Williams, M. R. 2004.** Cotton insect loss estimates – 2003, pp. 1258-1312. *In Proc. 2004 Beltwide Cotton Conf.*, San Antonio, TX. January 5-9, 2004.
- Williams, M. R. 2005.** Cotton insect loss estimates – 2004, pp. 1105-1160. *In Proc. 2005 Beltwide Cotton Conf.*, New Orleans, LA. January 4-7, 2005.
- Williams, M. R. 2006.** Cotton insect losses – 2005, pp. 1151-1204. *In Proc. 2006 Beltwide Cotton Conf.*, San Antonio, TX. January 3-6, 2006.
- Williams, M. 2007a.** Cotton insect loss estimates – 2006, pp. 1027-1042. *In Proc. 2007 Beltwide Cotton Conf.*, New Orleans, LA. January 9-12, 2007.
- Williams, M. 2007b.** Cotton insect losses – 2006, pp. 974-1026. *In Proc. 2007 Beltwide Cotton Conf.*, New Orleans, LA. January 9-12, 2007.
- Williams, M. R. 2008.** Cotton insect losses – 2007, pp. 927-979. *In Proc. 2008 Beltwide Cotton Conf.*, Nashville, TN. January 8-11, 2008.
- Williams, M. R. 2009.** Cotton insect losses – 2008, pp. 897-940. *In Proc. Beltwide Cotton Conf.*, San Antonio, TX. January 5-8, 2009.
- Williams, M. R. 2010a.** Cotton insect loss estimates – 2009, pp. 1099-1110. *In Proc. Beltwide Cotton Conf.*, New Orleans, LA. January 4-7, 2010.
- Williams, M. R. 2010b.** Cotton insect losses – 2009, pp. 1029-1073. *In Proc. Beltwide Cotton Conf.*, New Orleans, LA. January 4-7, 2010.
- Wiseman, B. R., W. P. Williams, and F. M. Davis. 1981.** Fall armyworm: resistance mechanisms in selected corns. *J. Econ. Entomol.* 74: 622-624.

- Wiseman, B. R., F. M. Davis,, and W. P. Williams. 1983.** Fall armyworm: larval density and movement as an indication of nonpreference in resistant corns. *Protec. Ecol.* 5: 135-141.
- Wiseman, B. R. and F. M. Davis. 1990.** Plant resistance to insects attacking corn and grain sorghum. *Florida Entomol.* 73: 446-458.
- Wittwer, S. H. 1995.** Food, Climate, and Carbon Dioxide: the Global Environment and World Food Production. CRC Press. 236 pp.
- Wood, K. A. 1979.** The influence of host plant on the susceptibility of the fall armyworm to insecticides. Thesis: Dept. of Entomology, Louisiana State University and Agricultural and Mechanical College. 29 pp.
- Wood, K. A., B. H. Wilson, and J. B. Graves. 1981.** Influence of host plant on the susceptibility of the fall armyworm to insecticides. *J. Econ. Entomol.* 74: 96-98.
- Yu, S. J. 1983.** Age variation in insecticide susceptibility and detoxification capacity of fall armyworm (Lepidoptera: Noctuidae) larvae. *J. Econ. Entomol.* 76: 219-222.
- Yu, S. J. 1992.** Detection and biochemical characterization of insecticide resistance in fall armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 85: 675-682.

CHAPTER 3

FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) DEVELOPMENT, SURVIVORSHIP, AND DAMAGE ON COTTON PLANTS EXPRESSING INSECTICIDAL PLANT-INCORPORATED PROTECTANTS

Introduction

First generation transgenic cotton, *Gossypium hirsutum* (L.), lines expressing plant-incorporated protectants, as *Bacillus thuringiensis* (Bt) Berliner insecticidal proteins, were introduced in the southern U.S. during 1996. Since then, adoption of Bt cotton has greatly increased because of high efficacy and ease-of-use as a pest management tool. United States Bt cotton acreage has increased across the cotton belt from an initial adoption of 12% in 1996 to 64% of the total cotton acreage in 2009 (Williams 1997, 2010). This high level of Bt cotton implementation has significantly decreased the use of foliar insecticides to control the primary Lepidopteran pests of cotton, reducing insecticide sprays by 2.0-5.5 applications per hectare across the southern U.S. (Edge et al. 2001).

The first Bt cotton technology, Bollgard[®] (Monsanto, St. Louis, MO), expressed a single insecticidal crystal (Cry) protein (Cry1Ac). This technology has now been phased out of commercial cotton production in the U.S. and is no longer registered for use. The efficacy of Bollgard[®] was limited in its target spectrum, but provided excellent control of primary lepidopteran pests including tobacco budworm, *Heliothis virescens* (F.), and pink bollworm, *Pectinophora gossypiella* (Saunders). New Bt cotton cultivars have been developed that express multiple insecticidal proteins such as Bollgard II[®] (Cry1Ac + Cry2Ab) (Monsanto, St. Louis, MO) and WideStrike[™] (Cry1Ac + Cry1F) (Dow AgroSciences, Wilmington, DE). These cultivars express “pyramided” Cry proteins (multiple proteins expressed within the same plant)

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and demonstrate higher efficacy against bollworm and many secondary lepidopteran pests when compared to the single protein expressed in Bollgard[®] cultivars (Gore et al. 2001, Chitkowski et al. 2003, Tindall et al. 2009).

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is one of several occasional cotton lepidopteran pests across the Southern U.S. This pest does not overwinter in cotton-producing regions, but instead migrates into these areas each year from the Gulf Coast and the Caribbean Islands (Luginbill 1928, Sparks 1979, Knipling 1980, Adamczyk et al. 1998). Therefore, annual infestations are unpredictable and difficult to detect prior to population establishment in cotton fields.

Fall armyworm infestations in cotton usually occur sporadically across production regions and within fields. Adults typically oviposit egg masses (10-500 eggs) in the lower two-thirds of the plant canopy on the abaxial (underside) surface of leaves. Larvae typically exhibit gregarious behavior immediately following eclosion as neonates and feed on the leaf near the site of oviposition. Later instars (>L2 stage) disperse both vertically within the plant canopy and horizontally to neighboring plants within a row (Ali et al. 1989, 1990).

Larval preference for plant tissue in the lower portion of the canopy generally makes control of this pest difficult to achieve with foliar insecticides because of poor spray deposition (Reed and Smith 2001). Older larvae have a tendency to feed within reproductive structures where they are further protected from applications of insecticides. To further increase control problems, larvae have an increasingly higher tolerance to insecticide exposure as they age (Yu 1983, Mink and Luttrell 1989).

Given the inherent difficulties in effective management of fall armyworm with conventional chemical control strategies, it is important to explore alternative methods of

control. Luttrell et al. (1999) demonstrated that fall armyworm was less susceptible to Cry1Ac (expressed in Bollgard[®] cotton) in diet assays compared to other primary pests of cotton such as tobacco budworm and bollworm. However, incomplete control of bollworm and the emergence of secondary lepidopteran pests in Bollgard[®] cotton have created the need for “pyramided” Bt lines such as Bollgard II[®] and WideStrike[™]. Although limited field studies have indicated that these technologies provide better efficacy against fall armyworm compared to Bollgard[®], there has been little evidence showing the overall effects of these technologies on fall armyworm development, survivorship, and associated damage to cotton plants.

The objective of these studies was to evaluate the effectiveness of the proteins in Bollgard[®], Bollgard II[®], and WideStrike[™] cotton lines against fall armyworm in field and laboratory trials. The field trials evaluated fall armyworm survivorship and subsequent plant injury on Bt and non-Bt cotton from artificial infestations. The laboratory studies characterized insect development on selected fruiting forms of these Bt cotton technologies. This knowledge of insect development and survivorship on Bt plants can be important in the design of insecticide resistance management (IRM) strategies. Therefore, results generated from this study should provide useful background information to develop other Bt cotton technologies for managing fall armyworm.

Materials and Methods

Fall Armyworm Colony Establishment and Maintenance. The fall armyworm colony (LSU-FAW) used at the Macon Ridge Research Station (MRRS) in this study originated from a field collection on cotton plants near Winnsboro, LA during 2005 and was supplemented with insects from field corn, *Zea mays* L., in the same area during 2006 and 2008. The colony (MS-FAW) used at the Southern Insect Management Research Unit (SIMRU) was originally collected

from non-Bt field corn in Washington County, MS during 2007 and has not been supplemented with wild populations. Each of the colonies was independently validated as the corn strain of fall armyworm using mitochondrial markers (Unpublished communication, R. Nagoshi, USDA-ARS, Gainesville, FL, Nov. 2008). The colony has been maintained in the laboratory on meridic diet (Stonefly Heliiothis Diet, Ward's Natural Science, Rochester, NY) using the methods as described in Adamczyk et al. (1998).

For each test (replicate), a cohort of 50 healthy pupae were removed from the colony, placed into plastic buckets (2.79 liters), and covered with cheesecloth. Upon adult eclosion, moths were fed a 10% sugar:water solution and allowed to mate. Eggs on cheesecloth sheets were allowed to eclose and larvae were reared on meridic diet until reaching the size needed for experiments.

Artificial Infestations in Field Trials. Field studies were conducted at the MRRS near Winnsboro, LA in Franklin Parish (32°8'8"N 91°41'23"W) and at the SIMRU near Stoneville, Mississippi in Washington County (33°25'23"N 90°53'36"W) during 2009 and 2010 (LA only). The cotton technologies tested each year included Bollgard[®] (Cry1Ac), Bollgard II[®] (Cry1Ac + Cry2Ab), WideStrike[™] (Cry1Ac + Cry1F), and a conventional non-Bt (Table 3.1). Three to four blocks of each variety were planted on sequential planting dates each year to provide continuous availability of cotton fruiting forms throughout the production season.

Infestation procedures were similar to those described by Gore et al. (2000) for caging bollworm larvae on white flowers and bolls. For each in-field infestation event (replicate), a single third-instar fall armyworm (≈ 7 d old; 30-45 mg/larva) was placed on a sympodial branch first-position cotton square (floral bud) (0.5-1.25 cm in diameter), white flower (anthesis), or boll (\approx quarter size; 200 accumulated heat units) of a randomly selected plant (only one infested

structure per plant). The larva and fruiting form were then enclosed within a nylon mesh exclusion cage (Mosquito Netting White Fabric, Hancock Fabrics, Baldwin, MS) which was sealed with a drawstring to prevent larval escape and reduce mortality from predators. Fruiting form infestations were initiated once plants had begun flowering and concluded when plants developed to “cutout” (beginning of physiological maturity, five or fewer main stem nodes above a first position sympodial white flower [NAWF < 5]). A single square on thirty plants was infested during each infestation event (replicate). At 2-3 d after infestation (DAI), all caged squares were visually inspected to record corolla penetration as well as larval survival. Infestations for white flowers and bolls were conducted similarly to those for squares. At 5-6 DAI, all caged white flowers and bolls were visually inspected to record carpel penetration and larval survival. The chosen endpoints of 2-3 DAI for squares and 5-6 DAI for white flowers and bolls were chosen based upon consumption of available plant material by infested larvae in preliminary trials.

Table 3.1. Cotton varieties used for field and laboratory studies, 2009-2010.

Cotton line	Protein(s)
PHY 425 RF ^a	non-Bt
DP 555 BG/RR ^b	Cry1Ac
STV 4554 B2/RF ^c	Cry1Ac + Cry2Ab
PHY 485 WRF ^d	Cry1Ac + Cry1F

^a Roundup Ready Flex; Phytogen Cottonseed, Dow AgroSciences, Indianapolis, IN.

^b Bollgard[®]; Roundup Ready; Delta & Pine Land Co., Monsanto Co., St. Louis, MO.

^c Bollgard II[®]; Roundup Ready Flex; Stoneville Pedigreed Seeds, Bayer CropScience, Research Triangle Park, NC.

^d WideStrike[™]; Roundup Ready Flex; Phytogen Cottonseed, Dow AgroSciences, Indianapolis, IN.

These data were analyzed using a one-way analysis of variance with PROC MIXED (SAS Institute 2004). Individual data was averaged for a single treatment value within a replication. Means were estimated using the LSMEANS statement and subjected to Dunnett’s

method for comparing treatments (Bt) to a standard (non-Bt) (SAS Institute 2004). Percent data were transformed using the arcsine square-root transformation before analysis (Zar 1999); however, actual means are presented in the results. The results for Bollgard[®], Bollgard II[®], and WideStrike[™] cotton lines were not statistically compared to each other to maintain compliance with contractual requirements from participating seed companies. Results for Bollgard[®], Bollgard II[®], and WideStrike[™] were independently compared to the non-Bt control.

No-choice Fresh Tissue Laboratory Bioassays. During 2007-2010, third-instar fall armyworms (30-45 mg/larva) were offered fresh cotton tissue (squares, white flowers, or bolls) removed directly from plants in field plots at the Winnsboro location. First-position fruiting forms (squares, white flowers, or bolls) were chosen from randomly selected plants within each plot, removed from the plant, and transported immediately back to the laboratory. Laboratory studies were initiated on flowering stage plants and concluded when plants matured to “cutout”. Larvae were placed individually into plastic cups (Solo Cup Co., Lake Forest, IL) for squares (30 ml) and bolls (96 ml) or jars for white flowers (118 ml; Uline, Pleasant Prairie, WI). Each respective container included two to three squares, a single white flower, or a single boll. Growth stages of fruiting forms at the time of field collection were equivalent to those previously described for field trials. Cotton tissue was evaluated daily and replaced as needed, but all survivors were offered fresh tissue at least every three days. Twenty to thirty larvae were infested for each treatment and fruiting form combination within a replication.

Development and survivorship of fall armyworm was recorded daily until all insects had either died or completed pupal development and emerged as adults. A larva was considered dead if it was incapable of movement after being placed on its dorsal surface and prodded with a camel-hair paintbrush. Treatments were compared based upon measurements of third instar-to-

pupa survivorship, larval duration, pupal weight (2008-2010 tests), pupal duration, third instar-to-adult survivorship, and damage to fruiting forms (squares and bolls only). Daily larval mortality values were used to calculate median lethal time (LT_{50} ; days required to reach 50% mortality) for each cotton line by structure combination (Morris 1988).

These data were analyzed using a one-way analysis of variance with PROC MIXED (SAS Institute 2004). Means were estimated using the LSMEANS statement and subjected to Dunnett's method for comparing treatments (Bt) to the standard (non-Bt cotton line), however, actual means are presented in the results (SAS Institute 2004). Median lethal time (LT_{50}) data were analyzed with probit analysis using Polo-Plus (LeOra Software 2006). A lack of overlap of 95% confidence limits was used to determine significant differences for fall armyworm survivorship between each Bt line and the non-Bt line. For the reasons previously described, results were not compared between the Bt technologies.

Results

Artificial Infestations in Field Trials. No significant differences ($P \geq 0.05$) were observed for any of the variables evaluated for fall armyworm larvae caged on Bollgard[®] squares compared to those caged on non-Bt squares at MRRS or SIMRU (Tables 3.2 and 3.3). At SIMRU, fewer survivors were recovered on Bollgard II[®] squares (1.5-fold) than on non-Bt squares. Fall armyworm larvae also damaged fewer Bollgard II[®] squares, 1.4 and 1.7-fold, compared to non-Bt squares at MRRS and SIMRU, respectively. Infestations of fall armyworm on WideStrike[™] squares resulted in a 1.8 and 1.9-fold decrease in the number of larvae recovered compared to infestations on non-Bt squares at MRRS and SIMRU, respectively. Fall armyworm damaged squares were 3.5 and 2.3-fold greater for the non-Bt cotton lines compared to WideStrike[™] cotton lines at MRRS and SIMRU, respectively.

Table 3.2. Survivorship and damage (mean \pm SEM) of fall armyworm (LSU-FAW) third instars caged on non-Bt and Bt cotton squares, white flowers, and bolls in field infestations, Winnsboro, LA, 2009-2010.

Fruiting Form ^a	Cotton Trait ^b	Alive ^c	Damage ^d
Squares	Non-Bt	70.6 \pm 6.3	77.3 \pm 4.5
	Bollgard	67.3 \pm 2.8	77.3 \pm 3.6
	Bollgard II	63.1 \pm 6.6	56.4 \pm 7.8*
	WideStrike	38.7 \pm 9.9*	22.0 \pm 6.1*
	df	3, 27	3, 27
	F	5.55	19.91
	P	0.0042	<0.0001
White Flowers	Non-Bt	47.0 \pm 5.9	68.1 \pm 10.2
	Bollgard	62.8 \pm 5.4	71.4 \pm 7.1
	Bollgard II	36.4 \pm 5.5	62.8 \pm 6.5
	WideStrike	14.0 \pm 5.2*	21.9 \pm 5.6*
	df	3, 21	3, 21
	F	13.80	8.40
	P	<0.0001	0.0007
Bolls	Non-Bt	56.7 \pm 4.0	59.7 \pm 5.9
	Bollgard	66.8 \pm 4.9	68.1 \pm 3.5
	Bollgard II	55.3 \pm 6.6	58.2 \pm 5.4
	WideStrike	15.1 \pm 6.1*	19.4 \pm 8.6*
	df	3, 24	3, 24
	F	30.79	21.26
	P	<0.0001	<0.0001

Means within columns followed by an asterisk (*) are significantly different from the corresponding non-Bt ($P=0.05$, Dunnett's).

^a Fruiting forms evaluated 2-3 DAI (squares) and 5-6 DAI (white flowers and bolls).

^b Cotton varieties: non-Bt = Phytogen 425 RF; Bollgard[®] = DP 555 BG/RR; Bollgard II[®] = Stoneville 4554 B2RF; and WideStrike[™] = Phytogen 485 WRF.

^c Percent of larvae recovered alive.

^d Percent of fruiting forms with outer wall penetrated by larvae.

Fall armyworm larval survivorship and damage on Bollgard[®] or Bollgard II[®] white flowers did not differ from those caged on non-Bt white flowers at MRRS (Table 3.2). At SIMRU, 2.0-fold fewer larval survivors were recovered on Bollgard II[®] white flowers than on non-Bt white flowers (Table 3.3). In addition, fall armyworm damaged white flowers were 1.6-fold greater on non-Bt white flowers compared to that on Bollgard II[®] white flowers at SIMRU.

Significantly fewer survivors were recovered on WideStrike™ white flowers (3.4 and 3.2-fold) compared to infestations on non-Bt white flowers at MRRS and SIMRU, respectively. Also, fall armyworm larvae caused less damage to white flowers of WideStrike™ cotton lines (3.1 and 3.1-fold) than to non-Bt white flowers at MRRS and SIMRU, respectively.

Table 3.3. Survivorship and damage (mean ± SEM) of fall armyworm (MS-FAW) third instars caged on non-Bt and Bt cotton squares, white flowers, and bolls in field infestations, Stoneville, MS, 2009.

Fruiting Form ^a	Cotton Trait ^b	Alive ^c	Damage ^d
Squares	Non-Bt	74.7 ± 3.9	77.7 ± 5.3
	Bollgard	71.3 ± 2.6	68.7 ± 3.0
	Bollgard II	51.0 ± 4.2*	45.3 ± 2.3*
	WideStrike	39.0 ± 4.2*	33.3 ± 3.3*
	df	3, 6	3, 6
	<i>F</i>	51.75	54.60
	<i>P</i>	0.0001	<0.0001
White Flowers	Non-Bt	51.0 ± 2.0	79.0 ± 4.2
	Bollgard	40.0 ± 4.0	80.3 ± 3.3
	Bollgard II	25.3 ± 2.3*	50.0 ± 5.1*
	WideStrike	15.7 ± 3.0*	25.3 ± 2.3*
	df	3, 6	3, 6
	<i>F</i>	33.54	45.91
	<i>P</i>	0.0004	0.0002
Bolls	Non-Bt	51.3 ± 3.0	65.7 ± 3.0
	Bollgard	54.3 ± 1.3	61.0 ± 4.2
	Bollgard II	36.7 ± 2.0*	45.3 ± 3.9*
	WideStrike	25.7 ± 1.3*	22.3 ± 2.3*
	df	3, 6	3, 6
	<i>F</i>	33.42	116.79
	<i>P</i>	0.0004	<0.0001

Means within columns followed by an asterisk (*) are significantly different from the corresponding non-Bt ($P=0.05$, Dunnett's).

^a Fruiting forms evaluated 2-3 DAI (squares) and 5-6 DAI (white flowers and bolls).

^b Cotton varieties: non-Bt = Phytogen 425 RF; Bollgard® = DP 555 BG/RR; Bollgard II® = Stoneville 4554 B2RF; and WideStrike™ = Phytogen 485 WRF.

^c Percent of larvae recovered alive.

^d Percent of fruiting forms with outer wall penetrated by larvae.

No differences were observed for larvae caged on non-Bt and Bollgard[®] or Bollgard II[®] bolls at MRRS (Table 3.2). At SIMRU, no significant differences were observed for larvae caged on non-Bt and Bollgard[®] bolls (Table 3.3). However, significantly fewer survivors were recovered (1.4-fold) and larvae caused less damage (1.5-fold) on Bollgard II[®] bolls compared to that on non-Bt bolls at SIMRU. Significantly fewer larvae were recovered on WideStrike[™] bolls (3.8 and 2.0-fold less) than on non-Bt bolls at MRRS and SIMRU, respectively. Fall armyworm larvae damaged fewer WideStrike[™] bolls (3.1 and 2.9-fold less) than non-Bt bolls at MRRS and SIMRU, respectively.

No-choice Fresh Tissue Laboratory Bioassays. No significant differences ($P \geq 0.05$) were observed for any variables evaluated for fall armyworm larvae offered Bollgard[®] squares compared to those offered non-Bt squares (Table 3.4). Pupation, adult eclosion, and damaged squares were significantly reduced when fall armyworm larvae were offered Bollgard II[®] squares compared to those offered non-Bt squares. Larval exposure to WideStrike[™] squares resulted in differences in pupation, adult eclosion, and damaged squares compared to those exposed to non-Bt squares. Complete larval mortality (100%) was recorded for larvae on WideStrike[™] cotton squares; therefore, no statistical analysis was possible for larval duration, pupal weight, or pupal duration on this Bt trait.

No significant differences were observed for any variable evaluated for larvae offered Bollgard[®] white flowers compared to those offered non-Bt white flowers (Table 3.5). Bollgard II[®] white flowers significantly increased larval stadia, as well as significantly reducing pupation, pupal weight, and adult eclosion compared to larvae exposed to non-Bt white flowers. Larvae offered WideStrike[™] white flowers had significantly longer larval duration and lower rates of

Table 3.4. Fall armyworm (LSU-FAW) third instar development and survivorship (mean \pm SEM) on non-Bt and Bt cotton squares in no-choice bioassays, Winnsboro, LA, 2007-2009.

Life History	Non-Bt ^a	Bollgard	Bollgard II	WideStrike	<i>F</i>	df	<i>P</i>
Larval duration (d)	14.7 \pm 0.9	18.3 \pm 0.7	20.0 \pm ---- ^b	---- ^c	3.71	2, 2	0.212
Pupation (%)	54.9 \pm 9.5	17.8 \pm 14.4	1.7 \pm 1.7*	0.0*	11.11	3, 9	0.002
Pupal weight (mg)	122.1 \pm 9.3	114.2 \pm 25.5	61.3 \pm ---- ^b	---- ^c	4.74	2, 2	0.174
Pupal duration (d)	11.1 \pm 0.5	10.2 \pm 1.6	16.0 \pm ---- ^b	---- ^c	2.23	2, 2	0.310
Adult eclosion (%)	47.3 \pm 8.8	22.5 \pm 12.0	0.8 \pm 0.8*	0.0*	12.49	3, 9	0.002
Damaged squares ^d (%)	58.7 \pm 12.3	53.8 \pm 14.2	36.1 \pm 10.9*	19.3 \pm 10.2*	19.88	3, 9	<0.001

Means within rows followed by an asterisk (*) are significantly different from the non-Bt ($P=0.05$, Dunnett's).

^a Cotton varieties: non-Bt = Phytogen 425 RF; Bollgard[®] = DP 555 BG/RR; Bollgard II[®] = Stoneville 4554 B2RF; and WideStrike[™] = Phytogen 485 WRF.

^b No standard error calculated due to low sample size.

^c No insect survivorship.

^d Percentage of squares initially damaged (\approx 3 DAI).

pupation and adult eclosion compared to larvae offered non-Bt white flowers. No larvae successfully emerged as adults on WideStrike™ white flowers.

Pupal duration and adult emergence of fall armyworm on Bollgard® bolls was significantly lower compared to those offered non-Bt bolls (Table 3.6). Fall armyworm exposed to Bollgard II® bolls had significantly lower rates of pupation and adult eclosion compared to larvae on non-Bt bolls. The WideStrike™ trait significantly reduced pupation and adult eclosion with fewer damaged bolls compared to larvae offered non-Bt bolls.

Larval survivorship (d) on each cotton line and structure combination was used to calculate the time (d) for 50% mortality (LT₅₀; median lethal time) to occur (Table 3.7). These values ranged from 4.52 d to 29.38 d on cotton squares, 7.42 to 46.26 d on white flowers, and 4.42 to 29.96 on bolls. Fall armyworm larvae offered Bollgard®, Bollgard II®, and WideStrike™ squares had significantly lower LT₅₀ values than larvae on non-Bt squares. Larvae on white flowers also demonstrated lower LT₅₀ values on Bollgard II® and WideStrike™ lines compared to that on the non-Bt line. White flower LT₅₀ values for the non-Bt and Bollgard® lines were estimated because 50% mortality did not occur on either of these lines by the endpoint of the test. LT₅₀ values for fall armyworm larvae offered Bollgard®, Bollgard II®, and WideStrike™ bolls were lower than for those offered bolls from non-Bt plants.

Discussion

The results of the current study show that Bollgard®, Bollgard II®, and WideStrike™ differ in their effectiveness against fall armyworm when directly compared to this insect's response on non-Bt cotton. Bollgard® cotton lines were largely ineffective in reducing survivorship or preventing damage from fall armyworm compared to the non-Bt lines in field or laboratory studies. In previous studies involving artificial and natural infestations of fall

Table 3.5. Fall armyworm (LSU-FAW) development and survivorship (mean \pm SEM) on non-Bt and Bt white flowers in no-choice bioassays, Winnsboro, LA, 2007-2009.

Life History	Non-Bt ^a	Bollgard	Bollgard II	WideStrike	<i>F</i>	df	<i>P</i>
Larval duration (d)	12.2 \pm 0.4	12.4 \pm 2.6	14.9 \pm 0.8*	14.0 \pm ---- ^{b*}	16.70	3, 9	<0.001
Pupation (%)	70.0 \pm 7.6	67.5 \pm 12.5	60.0 \pm 2.9*	2.5 \pm 2.5*	38.79	3, 12	<0.001
Pupal weight (mg)	146.0 \pm 10.4	156.5 \pm 5.3	118.0 \pm 9.5*	147.8 \pm ---- ^b	6.81	3, 6	0.023
Pupal duration (d)	11.8 \pm 0.4	10.7 \pm 0.9	11.6 \pm 0.8	---- ^c	0.16	2, 8	0.851
Adult eclosion (%)	65.0 \pm 7.6	60.0 \pm 10.0	36.7 \pm 10.1*	0.0*	36.04	3, 12	<0.001

Means within rows followed by an asterisk (*) are significantly different from the non-Bt ($P=0.05$, Dunnett's).

^a Cotton varieties: non-Bt = Phytogen 425 RF; Bollgard[®] = DP 555 BG/RR; Bollgard II[®] = Stoneville 4554 B2RF; and WideStrike[™] = Phytogen 485 WRF.

^b No standard error calculated due to low sample size.

^c No insect survivorship.

armyworm, no differences were observed between Bollgard[®] and non-Bt plants in numbers of fall armyworm larvae recovered using a shake sheet or in visual records of larvae in white flowers, pink flowers, and whole plant searches (Adamczyk et al. 2001a, Chitkowski et al. 2003). Laboratory assays using second instars on white flowers have shown no significant difference in larval survivorship or successful pupation on Bollgard[®] compared to non-Bt cotton tissue (Stewart et al. 2001). Chitkowski et al. (2003) found no significant differences between survivorship of second instars on Bollgard[®] and non-Bt squares or bolls. However, lower survivorship was observed when fall armyworm larvae were offered Bollgard[®] leaves from plant terminals compared to non-Bt terminal leaves. This observation was expected because of the generally higher expression of Cry1Ac in the upper regions of Bollgard[®] plants (Adamczyk et al. 2001b), but fall armyworm typically does not feed at this site. Ali et al. (1990) found that only first and second instars prefer to feed on leaves, but predominately are found in the lower two-thirds of the plant canopy. Adamczyk and Gore (2004) also found no difference in fall armyworm neonate survivorship between Bollgard[®] and non-Bt squares in laboratory tests. However, in the present study, LT₅₀ values were significantly different for larvae offered Bollgard[®] fruiting forms compared to those offered non-Bt fruiting forms, which suggests sub-lethal effects on fall armyworm larvae.

Results from the present study with Bollgard II[®] cotton lines were variable and inconsistent in field and laboratory tests. Field studies showed minimal effects of Bollgard II[®] plant structures on fall armyworm survivorship compared to that on non-Bt plant structures at MRRS, but significant effects were detected at SIMRU. The disparity in results between the two locations could be attributed to the use of different fall armyworm colonies at each location. In addition, laboratory studies revealed generally lower survivorship and LT₅₀ values across all

Table 3.6. Fall armyworm (LSU-FAW) development and survivorship (mean \pm SEM) on non-Bt and Bt bolls in no-choice bioassays, Winnsboro, LA, 2007-2009.

Life History	Non-Bt ^a	Bollgard	Bollgard II	WideStrike	<i>F</i>	df	<i>P</i>
Larval duration (d)	12.9 \pm 0.6	13.4 \pm 0.4	14.6 \pm 0.5	15.0 \pm 3.0	2.04	3, 11	0.166
Pupation (%)	65.3 \pm 3.6	55.0 \pm 3.7	40.6 \pm 6.3*	1.11 \pm 0.7*	51.80	3, 15	<0.001
Pupal weight (mg)	169.4 \pm 4.9	170.6 \pm 8.8	160.8 \pm 11.4	115.8 \pm 24.3	1.92	3, 9	0.196
Pupal duration (d)	9.9 \pm 0.7	11.2 \pm 0.9*	9.8 \pm 0.8	22.0 \pm ---- ^b	7.08	3, 10	0.008
Adult (%)	51.4 \pm 4.0	36.9 \pm 4.9*	28.1 \pm 6.1*	0.6 \pm 0.6*	31.20	3, 15	<0.001
Damaged bolls ^c (%)	75.0 \pm 4.4	68.8 \pm 4.2	64.2 \pm 8.1	17.9 \pm 6.9*	23.45	3, 9	<0.001

Means within rows followed by an asterisk (*) are significantly different from the non-Bt ($P=0.05$, Dunnett's).

^a Cotton varieties: non-Bt = Phytogen 425 RF; Bollgard[®] = DP 555 BG/RR; Bollgard II[®] = Stoneville 4554 B2RF; and WideStrike[™] = Phytogen 485 WRF.

^b No standard error calculated due to low sample size.

^c Percent of bolls initially damaged (\approx 5 DAI).

fruiting forms, but only a significant decrease in damaged squares. This variability across locations suggests the importance of environmental effects on Cry protein toxicity to insects. Sivasupramaniam et al. (2008) found significant differences in Cry protein expression in different Bt cotton plant tissues with the lowest expression occurring in large leaves, calyxes, and bracts, and the highest expression in terminal leaves and ovules. Therefore, additional toxicity may occur from exposure to Bollgard II[®] fruiting forms for time periods greater than those evaluated in the present studies. Adamczyk et al. (2001a) and Chitkowski et al. (2003), using artificial and natural infestations, respectively, recovered similar numbers of fall armyworms on Bollgard II[®] and non-Bt plots in field tests. Second instars offered white flowers showed no differences in larval survivorship between Bollgard II[®] and non-Bt tissue, however, there was a significant delay in the rates of larval pupation on Bollgard II[®] (Stewart et al. 2001). In field trials using artificial infestations, Leonard et al. (2006) found no differences in 2-d-old fall armyworm survivorship or their ability to penetrate bolls 7 DAI between Bollgard II[®] white flowers and non-Bt white flowers; however, significant differences were observed in fall armyworm survivorship and penetrated bolls on Bollgard II[®] and non-Bt cotton lines when using 5-d-old larvae. Disparity between these reports and the present study may be explained by the difference in larval size chosen for infestation. The detrimental effects on larvae may also be multiplied if earlier instars are exposed to Bollgard II[®] or any Cry protein. Adamczyk et al. (2004) showed higher mortality of fall armyworm neonates offered mid- and lower-canopy leaves from Bollgard II[®] plants compared to those fed non-Bt leaves. This increase in activity could be attributed to differences in the type of tissue or larval age.

WideStrike[™] cotton lines reduced survival of fall armyworm, reduced subsequent injury, which resulted in lower LT₅₀ values, regardless of fruiting form. Results were largely consistent

Table 3.7. Time-mortality (LT₅₀) responses of fall armyworm (LSU-FAW) larvae (L3 stage; 30-45 mg) on non-Bt and Bt cotton fruiting forms in laboratory trials, Winnsboro, LA, 2007-2010.

Fruiting Form	Cotton Trait ^a	N ^b	LT ₅₀ ^c	95% C.L. ^{c,d}	Slope ± SE	χ ^e	df ^f
Squares	Non-Bt ^f	4284	29.38	27.85-31.17	2.20 ± 0.09	24.39	34
	Bollgard	4320	15.90	15.30-16.52	2.54 ± 0.09	22.13	34
	Bollgard II	4320	7.22	6.89-7.54	3.46 ± 0.10	21.93	34
	WideStrike	4320	4.52	4.33-4.71	5.72 ± 0.25	28.15	34
White flowers	Non-Bt	3700	46.26 ^g	41.67-52.49	1.72 ± 0.10	13.49	35
	Bollgard	3700	48.72 ^g	43.71-55.62	1.77 ± 0.11	15.35	35
	Bollgard II	3700	21.86	21.22-22.52	3.78 ± 0.13	33.31	35
	WideStrike	3700	7.42	7.10-7.73	4.22 ± 0.14	33.33	35
Bolls	Non-Bt	5440	29.96	27.92-32.44	1.61 ± 0.07	22.66	30
	Bollgard	5440	19.59	18.80-20.45	2.10 ± 0.08	22.65	30
	Bollgard II	5440	13.63	13.03-14.24	1.86 ± 0.06	9.62	30
	WideStrike	5440	4.42	4.07-4.75	3.52 ± 0.10	70.17*	30

^a Cotton varieties: non-Bt = Phytogen 425 RF; Bollgard[®] = DP 555 BG/RR; Bollgard II[®] = Stoneville 4554 B2RF; and WideStrike[™] = Phytogen 485 WRF.

^b Number of insect days (number insects X number d tested).

^c Median lethal time (d) (time to 50% mortality on cotton fruiting form).

^d Confidence Limits.

^e Chi square values (* denotes significant value).

^f Degrees of freedom.

^g Estimate of LT₅₀ values (50% mortality did not occur).

between field and laboratory studies for this technology in demonstrating efficacy compared to the non-Bt cotton lines. Adamczyk and Gore (2004) recorded lower fall armyworm neonate survivorship on WideStrike[™] terminal leaves and square tissue compared to those offered non-Bt tissues. Survivorship of fall armyworm neonates was also significantly reduced when larvae were offered leaves from both the mid- and lower-canopy of WideStrike[™] plants compared to those offered leaves from non-Bt plants (Adamczyk et al. 2004, Siebert et al. 2008). Against native field infestations, Siebert et al. (2008) found fewer fall armyworm larvae infesting squares, flowers, and bolls of WideStrike[™] plants compared to those of non-Bt plants. In laboratory assays, Tindall et al. (2009) found higher larval mortality and less plant injury for

second instars offered WideStrike™ leaf, square, and white flower tissue compared to those offered non-Bt tissue. Siebert et al. (2009) reported Cry1F expression, while greatest in mature leaves lower in the plant canopy, is expressed consistently among other plant parts (terminal leaves, squares, flowers, and bolls). These results are similar to those in the present study where WideStrike™ cotton lines had significant effects on fall armyworm development and survivorship across fruiting forms.

Bollgard® technology was not registered for use or approved for commercial cotton production in 2010. Evaluation of lines expressing only Cry1Ac in this work served to provide a reference for the activity of a single Cry protein against fall armyworm. The lack of significant effects from Cry1Ac-expressing cotton lines suggests that much of the toxicity in pyramided cotton lines against fall armyworm is dependent on more active proteins, such as species-specific activity or a higher titer of overall insecticidal proteins expressed in Bollgard II® (Cry2Ab) and WideStrike™ (Cry1F). The variable performance of Bollgard II® against fall armyworm in these studies indicates that the Cry2Ab protein may not be expressed at a sufficient level or is highly sensitive to the environment (Dong and Li 2007). The Cry1F protein expressed in WideStrike™ cotton lines appears to be sufficiently expressed, although some variation in expression may occur between different fruiting forms (Siebert et al. 2008), as indicated in the present study where larvae were capable of emerging as adults on WideStrike™ bolls. None of the Bt cotton lines in the present study were immune to fall armyworm damage and in some instances larvae were capable of pupating and emerging as adults. This observation indicates that supplemental insecticide applications may be required to achieve satisfactory control or reduce the impact of heavy and persistent fall armyworm infestations in pyramided Bt cotton production systems.

References Cited

- Adamczyk, J. J., Jr., J. Holloway, J. B. Graves, and B. R. Leonard. 1998.** Larval survival and development of the fall armyworm (Lepidoptera: Noctuidae) on normal and transgenic *Bacillus thuringiensis* (Bt) cotton. *J. Econ. Entomol.* 91: 539-545.
- Adamczyk, J. J., Jr., L. C. Adams, and D. D. Hardee. 2001a.** Field efficacy and seasonal expression profiles for terminal leaves of single and double *Bacillus thuringiensis* toxin cotton genotypes. *J. Econ. Entomol.* 94: 1589-1593.
- Adamczyk, J. J., Jr., D. D. Hardee, L. C. Adams, and D. V. Sumerford. 2001b.** Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1A(c) δ -endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *J. Econ. Entomol.* 94: 284-290.
- Adamczyk, J. J., Jr., and J. Gore. 2004.** Laboratory and field performance of cotton containing Cry1Ac, Cry1F, and both Cry1Ac and Cry1F (WideStrike®) against beet armyworm and fall armyworm larvae (Lepidoptera: Noctuidae). *Florida Entomol.* 87: 427-432.
- Adamczyk, J. J., Jr., S. Greenberg, J. S. Armstrong, W. J. Mullins, L. B. Braxton, R. B. Lassiter, and M. W. Siebert. 2004.** Evaluations of Bollgard®, Bollgard II®, and WideStrike® technologies against beet and fall armyworm larvae (Lepidoptera: Noctuidae). *Florida Entomol.* 91: 531-536.
- Ali, A., R. G. Luttrell, H. N. Pitre, and F. M. Davis. 1989.** Distribution of fall armyworm (Lepidoptera: Noctuidae) egg masses on cotton. *Environ. Entomol.* 18: 881-885.
- Ali, A., R. G. Luttrell, and H. N. Pitre. 1990.** Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. *Environ. Entomol.* 19: 1060-1067.
- Chitkowski, R. L., S. G. Turnipseed, M. J. Sullivan, and W. C. Bridges, Jr. 2003.** Field and laboratory evaluations of transgenic cottons expressing one or two *Bacillus thuringiensis* var. *kurstaki* Berliner proteins for management of Noctuid (Lepidoptera) pests. *J. Econ. Entomol.* 96: 755-762.
- Dong, H. Z. and W. J. Li. 2007.** Variability of endotoxin expression in Bt transgenic cotton. *J. Agron. Crop Sci.* 193: 21-28.
- Edge, J. M., J. H. Benedict, J. P. Carroll, and H. K. Reding. 2001.** Bollgard cotton: an assessment of global economic, environmental, and social benefits. *J. Cotton Science* 5: 121-136.
- Gore, J., B. R. Leonard, G. E. Church, J. S. Russell, and T. S. Hall. 2000.** Cotton boll abscission and yield losses associated with first-instar bollworm (Lepidoptera: Noctuidae) injury to nontransgenic and transgenic Bt cotton. *J. Econ. Entomol.* 93: 690-696.

- 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: 1445-1451.
- Leonard, B. R., K. V. Tindall, and K. D. Emfinger. 2006.** Fall armyworm survivorship and damage in Bollgard and Bollgard 2 cotton, pp. 1080-1084. *In* M. Huffman and D. Richter [eds.], Proceedings, Beltwide Cotton Conferences, 3-6 January 2006, San Antonio, TX. National Cotton Council of America, Memphis, TN.
- LeOra Software. 2006.** POLO-Plus. A user’s guide to Probit or Logit analysis. LeOra Software, Berkeley, CA.
- Knipling, E. F. 1980.** Regional management of the fall armyworm—a realistic approach? *Florida Entomol.* 63: 468-480.
- Luginbill, P. A. 1928.** The fall armyworm. USDA Tech. Bull. 34. U.S. Gov. Print Office.
- Luttrell, R. G., L. Wan, and K. Knighten. 1999.** Variation in susceptibility of Noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxins proteins and commercial formulations of *Bacillus thuringiensis*. *J. Econ. Entomol.* 92: 21-32.
- Mink, J. S., and R. G. Luttrell. 1989.** Mortality of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs, larvae and adults exposed to several insecticides on cotton. *J. Entomol. Sci.* 24: 563-571.
- Morris, O.N. 1988.** Comparative toxicity of delta-endotoxin and thuringiensin of *Bacillus thuringiensis* and mixtures of the two for the bertha armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 81: 135-141.
- Reed, J. T. and D. B. Smith. 2001.** Droplet size and spray volume effects on insecticide deposit and mortality of heliothine (Lepidoptera: Noctuidae) larvae in cotton. *J. Econ. Entomol.* 94: 640-647.
- SAS Institute. 2004.** SAS version 9.1 for Windows. SAS Institute, Cary, NC.
- Siebert, M. W., J. M. Babock, S. Nolting, A. C. Santos, J. J. Adamczyk, Jr., P. A. Neese, J. E. King, J. N. Jenkins, J. McCarty, G. M. Lorenz, D. D. Fromme, and R. B. Lassiter. 2008.** Efficacy of Cry1F insecticidal protein in maize and cotton for control of fall armyworm (Lepidoptera: Noctuidae). *Florida Entomol.* 91: 555-565.
- Siebert, M. W., T. G. Patterson, G. J. Gilles, S. P. Nolting, L. B. Braxton, B. R. Leonard, J. W. Van Duyn, and R. B. Lassiter. 2009.** Quantification of Cry1Ac and Cry1F *Bacillus thuringiensis* insecticidal proteins in selected transgenic cotton plant tissue types. *J. Econ. Entomol.* 102: 1301-1308.
- Sivasupramaniam, S., W. J. Moar, L. G. Ruschke, J. A. Osborn, C. Jiang, J. L. Sebaugh, G. R. Brown, Z. W. Shappley, M. E. Oppenhuizen, J. W. Mullins, and J. T. Greenplate.**

- 2008.** Toxicity and characterization of cotton expressing *Bacillus thuringiensis* Cry1Ac and Cry2Ab2 proteins for control of lepidopteran pests. *J. Econ. Entomol.* 101: 546-554.
- Sparks, A. N. 1979.** A review of the biology of the fall armyworm. *Florida Entomol.* 62: 82-87.
- 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: 752-760.
- Tindall, K. V., M. W. Siebert, B. R. Leonard, J. All, and F. J. Haile. 2009.** Efficacy of Cry1Ac:Cry1F proteins in cotton leaf tissue against fall armyworm, beet armyworm, and soybean looper (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 102: 1497-1505.
- Williams, M. R. 1997.** Cotton insect losses 1996. *In* C. P. Dugger and D. A. Richter [eds.], *Proceedings, Beltwide Cotton Conferences, 7-10 January 1997, New Orleans, LA.* National Cotton Council of America, Memphis, TN.
- Williams, M. R. 2010.** Cotton insect losses 2009. *In* S. Boyd, M. Huffman, and B. Robertson [eds.], *Proceedings, Beltwide Cotton Conferences, 4-7 January 2010, New Orleans, LA.* National Cotton Council of America, Memphis, TN.
- Yu, S. J. 1983.** Age variation in insecticide susceptibility and detoxification capacity of fall armyworm (Lepidoptera: Noctuidae) larvae. *J. Econ. Entomol.* 76: 219-222.
- Zar, J. 1999.** *Biostatistical analysis*, 4th ed. Prentice Hall, Upper Saddle River, NJ.

CHAPTER 4

FALL ARMYWORM OVIPOSITIONAL PROFILE ON COTTON PLANTS EXPRESSING WIDESTRIKE™, BOLLGARD®, AND BOLLGARD II® CRY PROTEINS

Introduction

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), infestations in cotton, *Gossypium hirsutum* (L.), usually occur sporadically across the southern U.S. production regions and even vary considerably among fields due to the migratory behavior and wide host range of this species. Adults generally begin to appear in May-June and typically oviposit eggs in masses (60-500 eggs / cluster) within the lower two-thirds of the plant canopy and on the abaxial (underside) surface of leaves (Dew 1913, Ali et al. 1989). Neonates typically exhibit gregarious behavior immediately following eclosion and feed on the leaf near the site of the egg mass. Later instars disperse both vertically within the canopy and horizontally to neighboring plants within a row (Ali et al. 1990).

Transgenic cottons, which express crystalline (Cry) Bt proteins, are the standard management strategy for primary lepidopteran pests including bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.). Bt cultivars currently account for >70% of the total southern U.S. cotton acreage (Williams 2010). However, the Cry1Ac protein expressed in Bollgard® (Monsanto, St. Louis, MO) cottons has limited efficacy against fall armyworm (Adamczyk et al. 2001, Adamczyk et al. 2008). Cotton cultivars expressing the Bollgard II® (Cry1Ac:Cry2Ab) (Monsanto, St. Louis, MO) and WideStrike™ (Cry1Ac:Cry1F) (Dow AgroSciences, Indianapolis, IN) traits became commercially available in 2003 and 2005, respectively. These lines, expressing pyramided Bt proteins, exhibit greater efficacy against fall armyworm than the single protein-expressing Bollgard® (Hardke et al. 2011). However, inherent differences in protein expression throughout the cotton plant profile are evident between the

technologies (Bollgard[®], Bollgard II[®], and WideStrike[™]) (Adamczyk et al. 2001, Adamczyk et al. 2008, Siebert et al. 2009). Differences in protein concentrations are influenced by temporal expression patterns and distribution among plant tissues. This variability, coupled with physical plant characteristics, may influence lepidopteran ovipositional behavior and ultimately larval survivorship. In addition, early instars generally are more susceptible to Bt proteins and insecticides. Therefore, effective control strategies could be impacted by the location of egg masses and larvae.

Management strategies for fall armyworm in cotton usually include reactive insecticide sprays using action thresholds based on the presence of egg masses or early (L1-L2 stage) instars. Foliar insecticide applications are most effective when they target eggs and small larvae (Andrews et al. 2010, Akin et al. 2010, Baldwin et al. 2010). Knowledge of oviposition habits on Bt cotton lines could be important by influencing field sampling protocols and management strategies for fall armyworm infestations.

Previous work characterized fall armyworm oviposition on conventional non-Bt cotton (Ali et al. 1989). Fall armyworm prefers to oviposit deep within the canopy of non-Bt cotton plants. This, in turn, makes scouting for this species particularly difficult, and any differences between cotton lines may make this procedure more rigorous. If fall armyworm oviposition on Bt cottons occurs in a predictable region of the cotton plant canopy, scouting recommendations can be developed that will increase the accuracy and efficiency of fall armyworm sampling and management. As these Bt traits continue to be more widely adopted across the cotton belt, behavioral influences on pest organisms such as fall armyworm should be documented. Currently, there is no information describing the influence of Bt proteins expressed in cotton plants on the ovipositional behavior of adult fall armyworms. Therefore, the objective of this

study was to describe and compare the effects of Bt and non-Bt cotton traits on fall armyworm oviposition.

Materials and Methods

Fall Armyworm Colony Establishment and Maintenance. The fall armyworm colony used in this study originated from a field collection on cotton plants near Winnsboro, LA during 2005 and was supplemented with collections from field corn, *Zea mays* L., in the same area during 2006 and 2008. The colony was validated as the corn strain of fall armyworm using mitochondrial markers (Unpublished communication, R. Nagoshi, USDA-ARS, Gainesville, FL). The colony has been maintained in the laboratory on meridic diet (Stonefly Heliiothis Diet, Ward's Natural Science, Rochester, NY) using the methods described in Adamczyk et al. (1998).

Field Site and Treatment Description. All trials were conducted near Winnsboro, Louisiana (Franklin Parish) during July and August of 2008 and 2009. Treatments of cotton cultivars included a conventional non-Bt, Phytogen 425 RF (Roundup Ready Flex; Phytogen Cottonseed, Dow AgroSciences); DP 555 BG/RR (Bollgard[®] [Cry1Ac]/Roundup Ready; Delta & Pine Land Co.); Stoneville 4554 B2RF (Bollgard II[®] [Cry1Ac:Cry2Ab]/Roundup Ready Flex; Stoneville Seeds, Bayer CropScience); and Phytogen 485 WRF (WideStrike[™] [Cry1Ac:Cry1F]/Roundup Ready Flex; Phytogen Seeds, Dow AgroSciences). Four blocks of each variety were planted on sequential planting dates each year to provide continuous availability of cotton plants throughout July to August. Four translucent cages, each measuring 0.66 by 0.66 by 1.42 m and covered with a nylon screen (32 mesh / 2.54 linear cm), were fixed in plots just prior to each oviposition period with a plant population of two plants per cage. Plants were examined prior to infestation to ensure that no native egg masses were present. The phenological stages of cotton plants were recorded at the time of infestation (Table 4.1). Studies

were initiated once plants had begun flowering (bolls present) and concluded when plants developed to “cutout” (beginning of physiological maturity) (main stem nodes above a first position sympodial white flower [NAWF] < 5). Insecticide sprays were applied to cotton as needed to prevent loss of fruiting forms due to insect infestation, but were not treated with any insecticide within seven days of study initiation.

Field Cage Infestations. For each infestation event (replicate), a cohort of 80 healthy pupae were removed from the laboratory colony, separated by sex, placed into 2.79-liter plastic containers, and covered with cheesecloth. Upon adult eclosion, moths were fed a 10% v/v sugar:water solution. Male and female moths were then combined into plastic containers at a ratio of 1:1 (10 moths total per bucket). Insects were held in these containers for two-three nights to ensure females had an opportunity to mate prior to infestation in field cages (Ali et al. 1989). Moths from a single container were released into the field cage containing plants of one of the four cotton lines. Moths were released at \approx 1900 hours (CDST) and were allowed to oviposit for two-three nights on plants within the cage.

Containers with a sugar:water solution were placed in the center of each cage to ensure an additional food source for the moths. The cage and moths were removed at 0800 (CDST) hours following the oviposition period. All plants were destructively sampled to locate egg masses. Total number of egg masses, their location on each plant (main stem node location, branch [sympodial / monopodial] node location, height above soil [cm], and plant structure [surface of leaves, petioles, squares, flowers, and bolls]), and the phenological characteristics of the plants were recorded (Figure 4.1). Plant structures with egg masses were collected, placed in plastic bags, and transported to the laboratory. The number of eggs per mass was estimated at 10x with a dissecting microscope.

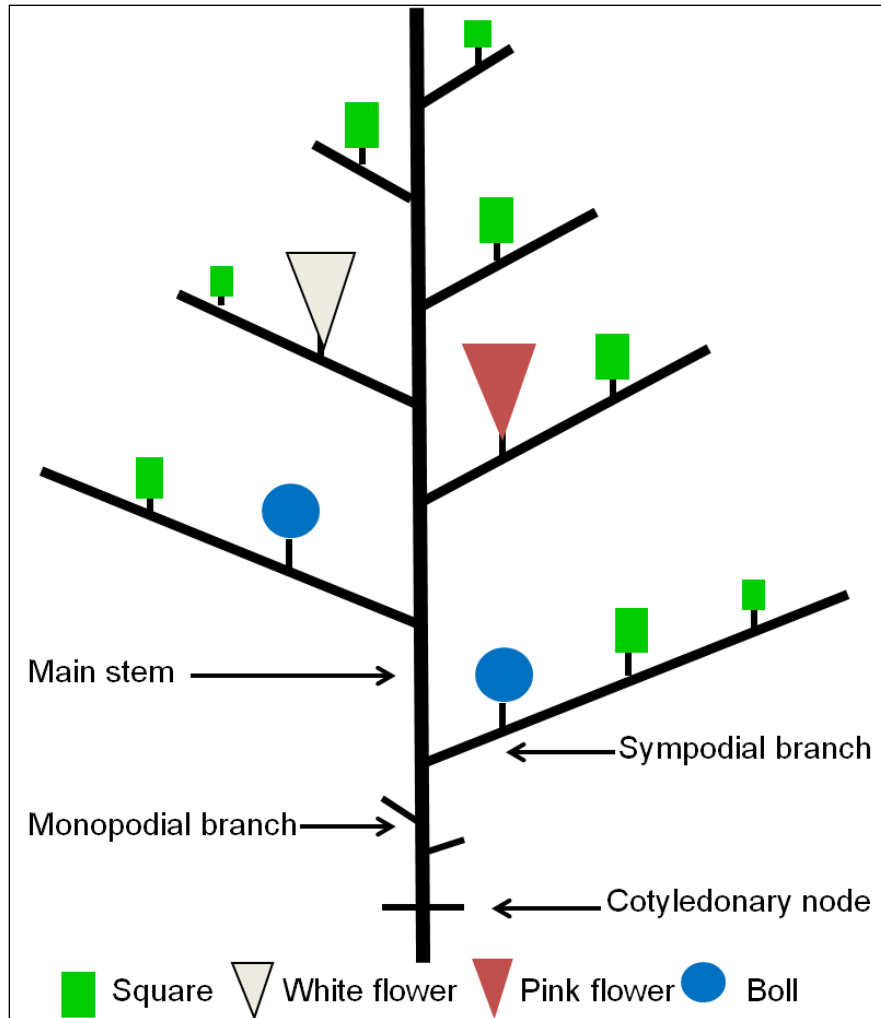


Figure 4.1. Cotton plant diagram.

Data Analysis. The entire experiment was repeated nine times for each cotton cultivar. Each egg mass represents an individual data point and was used to calculate mean statistics for each cultivar. All data were subjected to a one-way analysis of variance with PROC MIXED (SAS Institute 2004). Means for each treatment (cotton cultivar) were estimated using the LSMEANS statement; however, actual means are presented in the results. Where appropriate, data were then subjected to Dunnett's method for independently comparing results for selected treatments (cotton lines expressing Bt traits) to that of a standard control (non-Bt cultivar) (SAS

Institute 2004). This process of single comparison allows full compliance with contractual requirements from the participating seed industries for evaluating Bt traits in cotton lines.

Results

No significant differences were observed in number of main stem nodes or plant height among the cotton lines (Table 4.1). However, the Bollgard[®] cotton line had a greater number of monopodial branches and a higher first fruiting node on the main stem compared to the non-Bt cotton line. At the time of evaluation, plants of all cultivars averaged 107.7 ± 1.0 cm in height with an average of 19.2 ± 0.2 main stem nodes. In addition, plants had an average of 2.9 ± 0.1 vegetative branches, with the first main-stem fruiting node occurring on node 3.9 ± 0.1 .

Table 4.1. Phenological stages of non-Bt (conventional) and Bt cotton plants in Louisiana field cage trials during 2008-2009.

Plant characteristic	Cotton Line ^z			
	Non-Bt	Bollgard	Bollgard II	WideStrike
Main stem nodes	19.9 ± 0.3	19.3 ± 0.6	18.7 ± 0.2	18.5 ± 0.4
Plant height (cm) ^y	110.5 ± 1.4	105.3 ± 2.3	106.7 ± 1.6	108.4 ± 3.4
Monopodial branches	2.8 ± 0.2	$3.8 \pm 0.2^*$	2.2 ± 0.3	2.9 ± 0.3
First sympodial node	3.8 ± 0.2	$4.8 \pm 0.2^*$	3.2 ± 0.3	3.9 ± 0.3

^z Cotton varieties: non-Bt = Phytogen 425 RF; Bollgard[®] = DP 555 BG/RR; Bollgard II[®] = Stoneville 4554 B2RF; and WideStrike[™] = Phytogen 485 WRF.

^y Soil surface to first terminal leaf completely unfurled at the plant apex.

A total of 75 fall armyworm egg masses were collected on plants of all cotton cultivars during these trials (Table 4.2). Of these, 97.3% were deposited on leaves and 2.7% on petioles. More egg masses were observed on sympodial branch leaves (72.0%) compared to that (25.3%) on leaves emerging from the plant's main stem. The majority of the egg masses (86.3%) were deposited on abaxial surfaces of leaves. The number of eggs per mass ranged from 152.3 to 198.1 across all cultivars.

Table 4.2. Location and number (mean \pm SEM) of fall armyworm egg masses on non-Bt and Bt cotton plants in Louisiana field caging trials during 2008-2009.

Egg mass	Cotton Line ^z			
	Non-Bt	Bollgard	Bollgard II	WideStrike
Main stem node ^y	11.0 \pm 1.1	9.0 \pm 0.9	8.7 \pm 1.3	11.1 \pm 1.1
Branch node ^y	2.2 \pm 0.5	2.4 \pm 0.4	2.3 \pm 0.5	1.5 \pm 0.4
Abaxial leaf surface ^x	80.0 \pm 9.2	95.0 \pm 5.0	75.0 \pm 9.9	100
Height above soil (cm) ^y	75.5 \pm 5.5	61.0 \pm 4.9	66.5 \pm 5.8	80.0 \pm 5.5
Eggs per cluster ^y	198.1 \pm 26.4	159.7 \pm 27.7	152.3 \pm 23.0	168.6 \pm 34.3
Egg masses recovered ^w	2.33 \pm 0.4	2.33 \pm 0.7	2.22 \pm 0.9	1.44 \pm 0.6
Total egg masses	21	21	20	13

Means within columns followed by an asterisk (*) are significantly different from the corresponding non-Bt ($P=0.05$, Dunnett's).

^z Cotton varieties: non-Bt = Phytogen 425 RF; Bollgard[®] = DP 555 BG/RR; Bollgard II[®] = Stoneville 4554 B2RF; and WideStrike[™] = Phytogen 485 WRF.

^y Mean \pm SEM.

^x Percent \pm SEM.

^w Mean \pm SEM egg masses recovered per infestation event.

Differences were not detected ($P > 0.39$) between number of egg masses on the non-Bt and each Bt (Bollgard[®], Bollgard II[®], or WideStrike[™]) cultivar. The average main stem node location of egg masses ranged from nodes 8.7 to 11.1 across cultivars and the mean height of egg masses from the top of soil on each row ranged from 61.0 to 80.0 cm. Egg masses were found between sympodial branch nodes 1.5 to 2.4 on the main stem. Fall armyworm egg masses deposited on the abaxial leaf surface ranged from 75% to 100% across cultivars. Egg masses were predominately (>50%) deposited in the bottom two-thirds of the plant canopy, regardless of cultivar.

The occurrence of fall armyworm egg masses on plant main stem nodes (sympodial and monopodial) of cotton plants was sporadic throughout the plant canopy, but egg masses were predominately (>50%) deposited in the bottom two-thirds of the plant canopy, regardless of cultivar. In addition, no egg masses were recovered in the terminal area (uppermost two nodes)

of plants. Egg masses were observed on plant structures between main stem nodes 3 to 18 on the non-Bt cultivar (Figure 4.2). No more than two egg masses were found on structures at any common node. Two egg masses were found on plant main stem nodes 4, 8, 13, 14, 17, and 18. On Bollgard[®] cotton lines, egg masses were observed on main stem nodes 3 to 16. As many as three egg masses were found on structures of main stem nodes 7 and 12 for Bollgard[®] cotton. Egg masses deposited on Bollgard II[®] structures ranged from plant main stem nodes 2 to 18, with four egg masses on node 3 and three masses on node 5. Egg masses observed on WideStrike[™] structures ranged from main stem nodes 5 to 16. No more than two egg masses occurred on a structure located at an individual main stem node of WideStrike[™] structures, with two egg masses deposited on nodes 5, 10, 13, and 16.

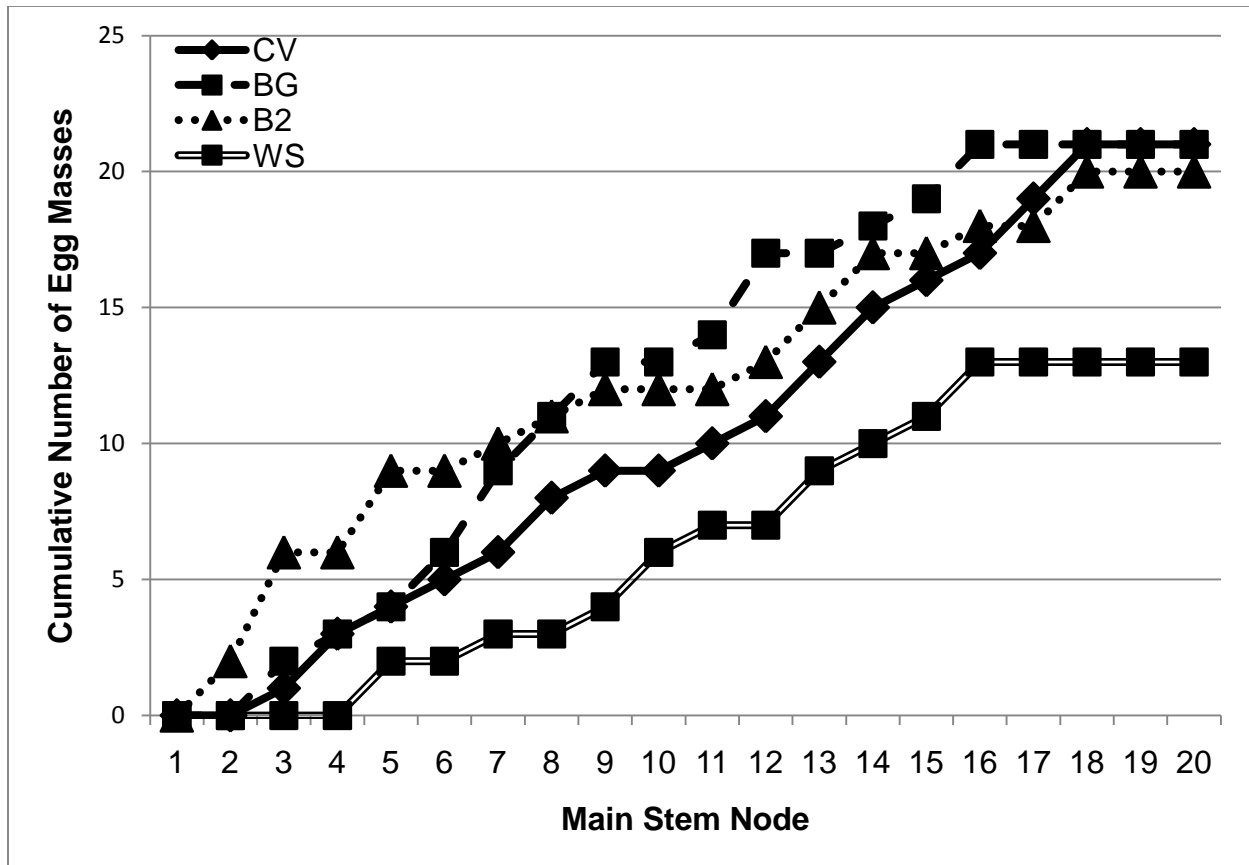


Figure 4.2. Cumulative frequency of fall armyworm egg mass deposition on main stem nodes of non-Bt and Bt cotton plants.

Discussion

The results under the conditions of the current study show that commercial Bt cotton cultivars (Bollgard[®], Bollgard II[®], and WideStrike[™]) do not alter fall armyworm oviposition behavior compared to that on a non-Bt cotton cultivar. Previous studies evaluating fall armyworm oviposition on pre-squaring non-Bt cotton plants documented that egg masses were found almost exclusively on leaves, and 100% of egg masses deposited on leaves were found on the abaxial surface (Pitre et al. 1983). Ali et al. (1989) found similar results when evaluating the distribution of fall armyworm egg masses on non-Bt cotton (ranging in age from pre-squaring to mature boll development). Fall armyworm adults deposited 95.9% of egg masses on leaves with 92.4% of those located on the abaxial leaf surface. The present study provides supporting data for earlier work by Pitre et al. (1983) and Ali et al. (1989) and illustrates fall armyworm does not deviate from its preference for depositing the majority of egg masses on the abaxial surface (underside) of leaves in the lower regions of the cotton canopy for all Bt cotton lines.

Ali et al. (1989) reported findings similar to those in the current study with mean main stem node locations of egg masses ranging from 7.8 (cotton with pink flowers) to 8.7 (cotton with mature bolls). The average sympodial and monopodial branch node location from the main stem reported by Ali et al. (1989) ranged from 1.9 to 2.0 on plants during the pink flowering and mature boll stages, respectively. The number of eggs per egg mass (104 to 207) was also similar during these stages. Previous research also has shown the majority of fall armyworm egg masses on non-Bt cotton to be in the lower half of the plant canopy (Ali et al. 1989), while in the current study the majority of egg masses were deposited in the lower two-thirds of the plant canopy.

The number of egg masses recovered per infestation event per cotton line (2.11) was seemingly low given the infestation of five females per cage. Simmons (1994) and Nagoshi

(2011) reported that female fall armyworm moths eclose earlier than males, which was also observed to be the case in this study. The delay in pairing males and females prior to infestation in cages may have negatively influenced the total number of eggs or egg clusters deposited by females. In an open field environment, it is likely that overlapping emergence of males and females within a generation provide for a longer period of effective mating which could result in higher rates of oviposition.

Conclusions

Fall armyworm adult behavior associated with oviposition on cotton plants was not affected by the proteins expressed in current commercially-available Bt cotton lines. None of the Bt cotton lines caused a significant change in the frequency or distribution of egg masses. These results provide support for the current scouting techniques of sampling and detecting fall armyworm infestations in cotton. The identification of fall armyworm infestations does not appear to be technology-dependent, reducing the rigor of an already difficult scouting process. With this knowledge, cotton pest managers should be able to rely on a single scouting method to identify and assess fall armyworm infestations in both Bt and non-Bt cotton fields.

Further study is needed to assess fall armyworm preference for cotton lines expressing different Bt technologies. The current study did not offer a choice of cotton cultivars and did not use insects that had been exposed to Bt cotton during prior generations. One area that remains unexplored is the potential for expression of Cry proteins in plant nectaries (floral and extra-floral) and the possible impact it may have on adult nutrition, reproduction, and oviposition. Cry proteins expressed in nectaries could have deleterious effects on foraging adults in much the same manner as those proteins expressed in vegetative and reproductive structures. Picard-Nizou et al. (1995) found that genetically modified oilseed rape, *Brassica napus* L., plants

exhibited changes in the quantity and sugar content of plant nectar. While these changes in plant biochemistry changes are not consistent across crops, the possibility exists that the Cry proteins expressed in Bt cotton plants could affect fall armyworm. Hardke et al. (2011) found WideStrike™ plants to be highly toxic to fall armyworm larvae, and there was a trend for fewer egg masses deposited on WideStrike™ plants than on non-Bt plants in the present study. Gore et al. (2002) reported differences in bollworm, *Helicoverpa zea* (L.), movement vertically within the plant canopy and higher numbers of infested white flowers and bolls for larvae on Bollgard® plants compared to non-Bt plants. Similar studies may be justified to further evaluate the influence of Bt cotton technologies on fall armyworm larval behavior and any subsequent effect on scouting and management techniques.

References Cited

- Adamczyk, J. J., Jr., J. Holloway, J. B. Graves, and B. R. Leonard. 1998.** Larval survival and development of the fall armyworm (Lepidoptera: Noctuidae) on normal and transgenic *Bacillus thuringiensis* (Bt) cotton. *J. Econ. Entomol.* 91: 539-545.
- Adamczyk, J. J., Jr., 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: 1589-1593.
- Adamczyk, J. J., Jr., S. Greenberg, J. S. Armstrong, W. J. Mullins, L. B. Braxton, R. B. Lassiter, and M. W. Siebert. 2008.** Evaluations of Bollgard®, Bollgard II®, and WideStrike® technologies against beet and fall armyworm larvae (Lepidoptera: Noctuidae). *Florida Entomol.* 91: 531-536.
- Akin, S., J. L. Bernhardt, J. Carson, J. D. Hopkins, K. Lofton, G. Lorenz, D. T. Johnson, T. J. Kring, P. J. McLeod, M. V. Meisch, P. Spradley, D. C. Steinkraus, G. Stuebaker, R. N. Wiedenmann, and J. Zawislak. 2010.** Cotton insect control. pp. 66-76. *In* G. Stuebaker (ed.) MP144 2010 Insecticide Recommendations for Arkansas. University of Arkansas Coop. Ext. Serv., Little Rock, AR.
- Ali, A., R. G. Luttrell, H. N. Pitre, and F. M. Davis. 1989.** Distribution of fall armyworm (Lepidoptera: Noctuidae) egg masses on cotton. *Environ. Entomol.* 18: 881-885.
- Ali, A., R. G. Luttrell, and H. N. Pitre. 1990.** Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. *Environ. Entomol.* 19: 1060-1067.

- Andrews, G., B. Burdine, A. Catchot, D. Cook, C. Daves, D. Dodds, J. Gore, R. Jackson, T. Koger, E. Larson, F. Musser, P. McKibben, J. Reed, J. Robbins, J. Smith, and S. Winters. 2010.** Cotton insect management. pp. 2-21. *In* A. Catchot (ed.) Insect Control Guide for Agronomic Crops 2010. Publ. 2471. Mississippi State Univ. Ext. Serv., Starkville, MS.
- Baldwin, J. L., L. D. Foil, M. L. Grodner, A. Hammond, G. Henderson, N. Hummel, S. Johnson, R. Leonard, A. Morgan, D. K. Pollet, J. Pyzner, T. E. Reagan, D. Reed, D. Ring, R. N. Story, and M. Stout. 2010.** Louisiana recommendations for control on cotton insects, pp. 121-126. *In* 2010 Louisiana Insect Pest Management Guide. Publ. 1838. Louisiana Coop. Ext. Serv., Baton Rouge, LA.
- Dew, J. A. 1913.** Fall army worm. *J. Econ. Entomol.* 6: 361-366.
- 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: 763-769.
- Hardke, J. T., R. E. Jackson, B. R. Leonard, and J. H. Temple. 2011.** Fall armyworm (Lepidoptera: Noctuidae) development, survivorship, and damage on cotton plants expressing insecticidal plant-incorporated protectants. *J. Econ. Entomol.* (Accepted: May 2011)
- Nagoshi, R. N. 2011.** Artificial selection for developmental rates in fall armyworm (Lepidoptera: Noctuidae) and its implications on the design of feeding studies. *Annals Entomol. Soc. Am.* 104: 88-94.
- Picard-Nizou, A. L., M. H. Pham-Delègue, V. Kerguelen, P. Douault, R. Marilleau, L. Olsen, R. Grison, A. Toppan, and C. Masson. 1995.** Foraging behaviour of honey bees (*Apis mellifera* L.) on transgenic oilseed rape (*Brassica napus* L. var. *oleifera*). *Transgenic Res.* 4: 270-276.
- Pitre, H. N., J. E. Mulrooney, and D. B. Hogg. 1983.** Fall armyworm (Lepidoptera: Noctuidae) oviposition: crop preferences and egg distribution on plants. *J. Econ. Entomol.* 76: 463-466.
- SAS Institute. 2004.** SAS version 9.1 for Windows. SAS Institute, Cary, NC.
- Siebert, M. W., T. G. Patterson, G. J. Gilles, S. Nolting, L. B. Braxton, B. R. Leonard, J. W. Van Duyn, and R. B. Lassiter. 2009.** Quantification of Cry1Ac and Cry1F *Bacillus thuringiensis* insecticidal proteins in various cotton plant tissue types containing Cry1Ac:Cry1F. *J. Econ. Entomol.* 102: 1301-1308.
- Simmons, A. M. 1994.** Effects of constant and fluctuating temperatures and humidities on the survival of *Spodoptera frugiperda* pupae (Lepidoptera: Noctuidae). *Florida Entomol.* 76: 333-340.

Williams, M. R. 2010. Cotton insect losses – 2009, p. 1029-1073. *In Proc. Beltwide Cotton Conf., New Orleans, LA. 4-7 Jan. 2010. Natl. Cotton Counc. Am., Memphis, TN.*

CHAPTER 5

OPPORTUNITIES TO MANAGE FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) ON BOLLGARD II[®] COTTON WITH REDUCED RATES OF INSECTICIDES

Introduction

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is an occasional, but often serious, pest of cotton, *Gossypium hirsutum* (L.), across the Mid-Southern U.S. Annual infestations are unpredictable because fall armyworm migrates from the Gulf Coast region and the Caribbean Islands into U.S. cotton production areas each year (Luginbill 1928, Sparks 1979, Knipling 1980). In addition, fall armyworm larvae usually occur in the lower two-thirds of the plant canopy and can be difficult to detect prior to the establishment of high populations in cotton fields. The significance of this pest has been further enhanced by the inconsistent performance of foliar insecticide sprays and *Bacillus thuringiensis* Berliner (Bt) transgenic cotton lines (Adamczyk et al. 2001, Stewart et al. 2001).

Fall armyworm adults generally prefer to deposit eggs in the lower cotton plant canopy, although they may oviposit throughout the entire plant profile when high populations of adults occur. Early instars feed on leaves at the site of the egg mass before dispersing vertically within the plant canopy, as well as horizontally to adjacent plants (Ali et al. 1989, 1990). Late instars ($\geq 4^{\text{th}}$ instars) prefer to feed within bolls low in the canopy which further protects them from insecticide sprays and exposure to subsequent residues (Young 1979, Pitre 1986). Furthermore, broad-leaved crops such as cotton tend to reduce the efficiency of insecticide deposition low in the plant canopy (Reed and Smith 2001). In addition, fall armyworm larvae also become more tolerant to insecticides as larvae increase in age and size (Yu 1983, Mink and Luttrell 1989). This increase in insecticide tolerance makes controlling fall armyworm progressively more difficult because many infestations are not discovered until larvae develop to late instars.

Transgenic *Bacillus thuringiensis* (Bt) cotton cultivars, expressing crystalline (Cry) proteins, have become the standard management strategy for lepidopteran pests and are planted on approximately 70% of the total U.S. cotton acreage (Williams 2010). Bollgard[®] cotton technology (Monsanto, St. Louis, MO), introduced in 1996, expressed the Cry1Ac protein and was effective in controlling tobacco budworm, *Heliothis virescens* (F.), and pink bollworm, *Pectinophora gossypiella* (Saunders). However, this technology failed to provide complete control of other lepidopteran pests such as bollworm, *Helicoverpa zea* (Boddie), and fall armyworm. Insecticide applications were common and necessary to control these pests in Bollgard[®] cotton (Adamczyk et al. 2001, 2008). The success of Bollgard[®] in controlling tobacco budworm and pink bollworm did, however, lead to the development of additional Bt cotton lines which express pyramided Bt proteins, including Bollgard II[®] (Cry1Ac + Cry2Ab) (Monsanto, St. Louis, MO) and WideStrike[™] (Cry1Ac + Cry1F) (Dow AgroSciences, Indianapolis, IN) in 2003 and 2005, respectively. These pyramided Bt lines have demonstrated higher efficacy against bollworm and fall armyworm compared to Bollgard[®], but none of the traits offer immunity to injury from these pests (Chitkowski et al. 2003, Siebert et al. 2008, Hardke et al. 2011a).

During the past few years, there has been a need to control fall armyworm populations with supplemental foliar insecticide applications. Unfortunately, there have been no previous studies to examine the influence of the Bt proteins expressed in transgenic cotton lines on the effectiveness of insecticide applications for fall armyworm. Therefore, the objective of this study was to evaluate the effectiveness of reduced (one-half) rates of insecticides applied to Bollgard II[®] cotton lines compared to full rates applied to non-Bt (conventional) cotton lines in field trials. These trials evaluated insecticide efficacy against fall armyworm in those situations where insecticide coverage is sufficient (terminal area of the cotton plant) and where spray

coverage may be compromised (mid-canopy). Cotton insect control guides currently do not distinguish between conventional and Bt cottons in their recommendations for control of fall armyworm or other caterpillar pests (Akin et al. 2010, Andrews et al. 2010, Baldwin et al. 2010). Insecticide performance information on Bollgard II[®] cotton can be important in identifying chemical control recommendations for managing native fall armyworm infestations and can provide reference data for future studies of insecticide and Bt cotton interactions.

Materials and Methods

Fall Armyworm Colony Establishment and Maintenance. The fall armyworm colony used in this study originated from a field collection on cotton near Winnsboro, LA during 2005 and was supplemented with collections from field corn in the same area during 2006 and 2008. The colony was validated as the corn strain of fall armyworm using mitochondrial markers (Unpublished communication, R. Nagoshi, USDA-ARS, Gainesville, FL). The colony has been maintained in the laboratory on meridic diet (Stonefly Heliiothis Diet, Ward's Natural Science, Rochester, NY) using the methods previously described in Adamczyk et al. (1998).

Site and Treatment Description. Field studies were conducted at the Macon Ridge Research Station (MRRS) near Winnsboro, LA in Franklin Parish (32° 8' 8" N 91° 41' 23" W) and at the USDA-ARS Southern Insect Management Research Unit (SIMRU) near Stoneville, MS in Washington County (33° 25' 23" N 90° 53' 36" W) during 2010. Cotton lines included Phytogen 425 RF (non-Bt; Roundup Ready Flex; Phytogen Cottonseed, Dow AgroSciences, Indianapolis, IN) and Stoneville 4554 B2/RF (Bollgard II[®] [Cry1Ac + Cry2Ab]; Roundup Ready Flex; Stoneville Pedigreed Seeds, Bayer CropScience, Research Triangle Park, NC). Multiple blocks of each variety were planted at each site on sequential planting dates to provide adequate availability of plants.

Insecticide Application. Tests were initiated when quarter-size bolls (≈ 200 accumulated heat units) were common throughout lower cotton canopy and were terminated prior to plants developing to cutout (main stem nodes above a sympodial first position white flower [NAWF] = 5). Insecticides were applied on 30 August and 4 September at MRRS and on 17 and 28 September at SIMRU. Insecticides at MRRS were applied using a high-clearance sprayer and a CO₂-charged spray system calibrated to deliver 107.6 liters per ha through TX-8 hollow cone nozzles (Spraying Systems Company, Wheaton, IL). Insecticides at SIMRU were applied with a high-clearance sprayer and a CO₂-charged spray system calibrated to deliver 106.6 liters per ha through TXVS-12 cone jet nozzles. Insecticides included chlorantraniliprole, flubendiamide, lambda-cyhalothrin, novaluron, spinetoram, and a non-sprayed control (Table 5.1). Conventional cotton plots were sprayed with full recommended rates of insecticides while Bollgard II[®] plots were sprayed with one-half of the recommended rates (Table 5.1).

Larval Infestations on Cotton Leaves and Bolls. Fall armyworm larvae were infested on cotton plant tissue in a manner similar to that described by Tindall et al. (2006). Plant tissues were removed from field plots approximately one hour after treatment. Ten leaves (second fully expanded leaf; second node from top of plant; upper canopy) and ten bolls (\approx quarter-size; first position on a sympodial branch; mid-canopy [mean plant height ≈ 109 cm]) were collected from each plot and returned to the laboratory. A single third-instar (30-45 mg; 7-9 d old) was infested on an individual leaf or boll. Ten larvae were exposed to each treatment (cotton line and insecticide combination) on each infestation event (insecticide application). Larvae were evaluated at 3 d after infestation (DAI) on leaves and at 3 and 7 DAI on bolls. Two replications were conducted at each location (MRRS and SIMRU) for a total of four replications and 40 larvae on each treatment.

Table 5.1. Insecticide treatments evaluated in field studies on non-Bt and Bollgard II[®] cotton lines.

Common name	Trade name	Formulation (g/liter)	Insecticide Rates ^z		Class ^x	Manufacturer
			Non-Bt ^y (Full)	Bollgard II ^y (Reduced)		
chlorantraniliprole	Coragen	200 SC ^w	0.102	0.051	Diamide (28)	DuPont Crop Protection, Wilmington, DE
flubendiamide	Belt	480 SC	0.105	0.053	Diamide (28)	Bayer Crop Science, Research Triangle Park, NC
lambda-cyhalothrin	Karate Z	250 EC ^v	0.046	0.023	Pyrethroid (3A)	Syngenta Crop Protection, Greensboro, NC
novaluron	Diamond	100 EC	0.044	0.022	Benzoylurea (15) [IGR]	Makhteshim Agan of North America, Inc., Raleigh, NC
spinetoram	Radiant	120 SC	0.070	0.035	Spinosyn (5)	Dow AgroSciences, Indianapolis, IN

^z kg AI/ha; Louisiana Insect Pest Management Guide 2010,

http://www.lsuagcenter.com/en/crops_livestock/crops/Cotton/Insects/Louisiana+Insect+Pest+Management+Guide.htm.

^y Cotton varieties: non-Bt = Phytogen 425 RF and Bollgard II[®] = Stoneville 4554 B2RF.

^x IRAC International MoA Working Group 2010, http://www.irac-online.org/wp-content/uploads/2009/09/MoA-classification_v7.0.4-5Oct10.pdf.

^w Soluble concentrate.

^v Emulsifiable concentrate.

Data Analysis. Treatments (cotton line and insecticide combinations) were randomly arranged within each replication (spray date / infestation event). Larval mortality percentages were transformed (arcsine square-root) and subjected to a one-way analysis of variance with PROC GLM (SAS Institute 2004). Treatment means were then separated according to Fisher's Protected LSD (SAS Institute 2004). Means were transformed for analysis; however, actual non-transformed means are presented in the results.

Results

Differences were not detected for treatment (cotton line and insecticide) by location interaction for terminal leaves 3 DAI ($df = 11, 23; F = 1.66; P = 0.1471$), for bolls 3 DAI ($df = 11, 23; F = 1.08; P = 0.4198$), and for bolls 7 DAI ($df = 11, 23; F = 0.54, P = 0.8534$). Therefore, data for the two locations were combined. Fall armyworm mortality on non-sprayed Bollgard II[®] terminal leaves (7.5%) was not significantly greater than that observed on non-sprayed conventional leaves (0.0%). Fall armyworm mortality on insecticide-sprayed Bollgard II[®] leaves at 3 DAI ranged from 30.0 to 95.0% compared to 22.5 to 82.5% on insecticide-sprayed conventional leaves (Table 5.2). On Bollgard II[®] leaves sprayed with reduced insecticide rates, chlorantraniliprole, flubendiamide, lambda-cyhalothrin, and spinetoram caused significantly greater mortality ($P < 0.05$) than was observed on non-sprayed Bollgard II[®] leaves. Significantly higher fall armyworm mortality was observed on chlorantraniliprole, flubendiamide, and spinetoram-sprayed Bollgard II[®] leaves compared to that on novaluron-sprayed Bollgard II[®] leaves ($P < 0.05$). Full rates of chlorantraniliprole, flubendiamide, lambda-cyhalothrin, and spinetoram on conventional leaves caused significantly greater mortality than was observed on the non-sprayed control. In addition, chlorantraniliprole-sprayed, flubendiamide-sprayed, and spinetoram-sprayed conventional leaves had higher fall armyworm mortality than novaluron-

sprayed conventional leaves. Differences were not observed for fall armyworm mortality when comparing the reduced rate of an insecticide on Bollgard II[®] cotton to each respective insecticide at the full rate on conventional cotton ($P > 0.05$).

Table 5.2. Mortality (\pm SE) of fall armyworm third instars on non-Bt and Bollgard II[®] cotton terminal leaves 3 d after infestation.

Technology	Insecticide	Rates	% Mortality ^z
Bollgard II ^y (reduced rates)	chlorantraniliprole	0.051	90.0 \pm 10.0a
	flubendiamide	0.053	80.0 \pm 13.5abc
	lambda-cyhalothrin	0.023	55.0 \pm 9.6cd
	novaluron	0.022	30.0 \pm 17.8de
	spinetoram	0.035	95.0 \pm 2.9a
	non-treated	----	7.5 \pm 4.8ef
Non-Bt ^y (full rates)	chlorantraniliprole	0.102	82.5 \pm 11.8abc
	flubendiamide	0.105	67.5 \pm 19.7abc
	lambda-cyhalothrin	0.046	52.5 \pm 18.0bcd
	novaluron	0.044	22.5 \pm 11.1def
	spinetoram	0.070	82.5 \pm 17.5ab
	non-treated	----	0.0 \pm 0.0f
	df		11, 33
	<i>F</i>		8.63
	<i>P</i>		<0.0001

^z Means followed by the same letter are not significantly different according to Fisher's Protected LSD ($P = 0.05$).

^y Cotton varieties: non-Bt = Phytogen 425 RF and Bollgard II[®] = Stoneville 4554 B2RF.

No significant differences were detected for fall armyworm mortality on non-sprayed Bollgard II[®] bolls (2.5%) compared to that on non-sprayed conventional bolls (5.0%) at 3 DAI (Table 5.3). Insecticide-sprayed Bollgard II[®] bolls caused fall armyworm mortality ranging from 5.0 to 80.0% compared to 17.5 to 80.0% on conventional bolls at 3 DAI. On Bollgard II[®], mortality was higher on bolls sprayed with chlorantraniliprole and spinetoram compared to that on lambda-cyhalothrin-sprayed, novaluron-sprayed, and non-sprayed Bollgard II[®] bolls.

Flubendiamide-sprayed bolls also produced higher mortality than non-sprayed Bollgard II[®] bolls. Conventional bolls sprayed with full rates of chlorantraniliprole and spinetoram increased fall armyworm mortality compared to that on novaluron-sprayed and non-sprayed conventional bolls. Conventional bolls sprayed with spinetoram also had higher mortality ($P < 0.05$) compared to that on flubendiamide and lambda-cyhalothrin-sprayed conventional bolls. Fall armyworm mortality levels were similar for conventional bolls sprayed with full insecticide rates and Bollgard II[®] bolls sprayed with reduced rates of the same insecticide ($P > 0.05$).

Table 5.3. Mortality (\pm SE) of fall armyworm third instars on non-Bt and Bollgard II[®] cotton bolls 3 d after infestation.

Technology	Insecticide	Rates	% Mortality ^z
Bollgard II ^y (reduced rates)	chlorantraniliprole	0.051	70.0 \pm 23.8ab
	flubendiamide	0.053	42.5 \pm 17.0bcd
	lambda-cyhalothrin	0.023	17.5 \pm 10.3def
	novaluron	0.022	5.0 \pm 2.9ef
	spinetoram	0.035	80.0 \pm 14.1a
	non-treated	----	2.5 \pm 2.5f
Non-Bt ^y (full rates)	chlorantraniliprole	0.102	65.0 \pm 11.9abc
	flubendiamide	0.105	40.0 \pm 17.8bcde
	lambda-cyhalothrin	0.046	30.0 \pm 12.2cde
	novaluron	0.044	17.5 \pm 7.5def
	spinetoram	0.070	80.0 \pm 13.5a
	non-treated	----	5.0 \pm 2.9ef
	df		11, 33
	<i>F</i>		6.85
	<i>P</i>		<0.0001

^z Means followed by the same letter are not significantly different according to Fisher's Protected LSD ($P = 0.05$).

^y Cotton varieties: non-Bt = Phytogen 425 RF and Bollgard II[®] = Stoneville 4554 B2RF.

Fall armyworm mortality observed on Bollgard II[®] bolls (27.5%) did not significantly differ from that on non-sprayed conventional bolls (20.0%) at 7 DAI (Table 5.4). Fall

armyworm mortality on insecticide-sprayed Bollgard II[®] bolls at 7 DAI ranged from 55.0 to 100% and from 52.5 to 100% on conventional bolls. Fall armyworm mortality was higher on chlorantraniliprole, flubendiamide, novaluron, and spinetoram-sprayed Bollgard II[®] bolls than that on non-sprayed Bollgard II[®] bolls. Bollgard II[®] bolls sprayed with chlorantraniliprole, flubendiamide, and spinetoram also had significantly higher mortality compared to that on lambda-cyhalothrin-sprayed Bollgard II[®] bolls. Conventional bolls sprayed with chlorantraniliprole, flubendiamide, novaluron, and spinetoram significantly increased fall armyworm mortality above that on non-sprayed conventional bolls. Significantly higher fall armyworm mortality was observed on chlorantraniliprole-sprayed conventional bolls compared to that on lambda-cyhalothrin-sprayed and novaluron-sprayed conventional bolls. No significant differences were observed in fall armyworm mortality between full rates applied to conventional bolls and reduced rates on Bollgard II[®] bolls for the same insecticide ($P > 0.05$).

Individual insecticide efficacy against fall armyworm remained similar across conventional and Bollgard II[®] cotton lines, despite the use of reduced rates on Bollgard II[®]. Overall mortality values were significantly higher ($df = 1, 80$; $F = 15.13$; $P = 0.0002$) for larvae infested on terminal leaves (3 DAI) compared to those infested on bolls (3 DAI), which would be expected due to the adequate insecticide coverage achieved in the terminal area of the plant canopy. Fall armyworm mortality on non-sprayed Bollgard II[®] cotton terminal leaves and bolls was low and did not differ from that on non-sprayed conventional cotton for the same structure ($P > 0.05$).

Discussion

The Bollgard II[®] trait did not significantly reduce fall armyworm survivorship compared to that on conventional cotton under the conditions of the current study. Fall armyworm

survivorship from third-instar to pupation on Bollgard II[®] bolls (>40%) has been documented in recent laboratory studies (Hardke et al. 2011a). In addition, Hardke et al. (2011a) reported >55% third-instar survival on Bollgard II[®] bolls in field studies 5-6 DAI.

Table 5.4. Mortality (\pm SE) of fall armyworm third instars on non-Bt and Bollgard II[®] cotton bolls 7 d after infestation.

Technology	Insecticide	Rates	% Mortality ^z
Bollgard II ^y (reduced rates)	chlorantraniliprole	0.051	100.0 \pm 0.0a
	flubendiamide	0.053	97.5 \pm 2.5a
	lambda-cyhalothrin	0.023	55.0 \pm 18.5cde
	novaluron	0.022	75.0 \pm 10.4abc
	spinetoram	0.035	87.5 \pm 9.5ab
	non-treated	----	27.5 \pm 10.3de
Non-Bt ^y (full rates)	chlorantraniliprole	0.102	100.0 \pm 0.0a
	flubendiamide	0.105	72.5 \pm 17.0abc
	lambda-cyhalothrin	0.046	52.5 \pm 17.5cde
	novaluron	0.044	62.5 \pm 9.5bcd
	spinetoram	0.070	85.0 \pm 11.9ab
	non-treated	----	20.0 \pm 5.8e
	df		11, 33
	<i>F</i>		5.55
	<i>P</i>		<0.0001

^z Means followed by the same letter are not significantly different according to Fisher's Protected LSD ($P = 0.05$).

^y Cotton varieties: non-Bt = Phytogen 425 RF and Bollgard II[®] = Stoneville 4554 B2RF.

Insecticide toxicity to fall armyworm varied among products on both Bt and conventional cotton lines. The more newly-registered insecticides (chlorantraniliprole, flubendiamide, and spinetoram) generally proved to be more efficacious against fall armyworm than standard, commonly used products (lambda-cyhalothrin and novaluron) recommended against this pest. Field studies with these same insecticides against fall armyworm in grain sorghum showed efficacy levels similar to that in the present study, with newer products significantly reducing

infested whorls and exhibiting increased residual efficacy compared to standard insecticides (Hardke et al. 2011b). Smith and Catchot (2009) also reported a significant reduction in fall armyworm larvae on chlorantraniliprole-sprayed conventional corn plants compared to plants sprayed with novaluron, lambda-cyhalothrin, and the non-sprayed control. In addition, dose-mortality responses developed for these insecticides against fall armyworm in laboratory studies follow a similar trend in order of toxicity (Hardke et al. 2011b). The LC₅₀ values of these insecticides against fall armyworm, from most to least efficacious, were spinetoram, chlorantraniliprole, novaluron, flubendiamide, and lambda-cyhalothrin, respectively.

Few studies have examined the combined effects of Bt plants and foliar insecticide sprays against pests of field crops. Many of the Bt traits are highly effective against specific species, and additional foliar sprays are used for non-target pests. However, many species of Lepidoptera, either as primary or secondary pests, express a range of susceptibility to Bt traits in cotton and other field crops. These effects of the Bt traits become important when determining the need for supplemental insecticide sprays and actual selection of a treatment. Lynch et al. (1999) evaluated corn earworm damage to sweet corn ears on Bt and conventional corn hybrids either non-sprayed or sprayed with one, three, or five insecticide applications. Non-sprayed Bt hybrids were successful in reducing corn earworm damaged ears compared to non-sprayed conventional hybrids. Foliar insecticide sprays on Bt hybrids further reduced the incidence and severity of corn earworm damage to ears compared to Bt hybrids receiving no foliar insecticide applications and sprayed conventional hybrids. Insecticide rate was not evaluated in this study, but Lynch et al. (1999) were able to establish the usefulness of combining Bt hybrids and insecticide sprays for management of a target pest.

In some instances, Bt crops alone can provide sufficient efficacy and reduce the potential benefits of a supplemental insecticide spray. Cooper et al. (2006) evaluated Colorado potato beetle survivorship and damage on conventional and Bt potatoes with and without an insecticidal protein (avidin) in laboratory experiments. Insecticide treatment did not significantly affect insect survivorship and plant damage on Bt potatoes due to the low larval survival on non-treated Bt potatoes. Bommireddy and Leonard (2008) reported extremely low survivorship of bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), on pyramided cotton lines expressing Vip3A and Cry1Ab proteins. Vip3A + Cry1Ab plants sprayed with an insecticide may not significantly reduce bollworm or tobacco budworm survivorship below that on non-sprayed Vip3A + Cry1Ab plants. The value of foliar sprays on Bt crops should be evaluated for each pest, crop, and Bt trait(s).

Limited information currently exists on the interactions between cotton lines expressing Bt proteins and foliar insecticides for control of Lepidopteran pests. Jackson et al. (2003, 2005) evaluated insecticide sprays on Bollgard II[®] cottons for bollworm control. Damaged bolls and larval survivors were reduced 9.5-fold and >2,000-fold, respectively, in insecticide-sprayed Bollgard II[®] plots compared to non-sprayed Bollgard II[®] plots (Jackson et al. 2003). Insecticide-sprayed Bollgard II[®] plots had fewer damaged fruiting forms (flower buds [squares] and bolls) and higher seedcotton yields compared to non-sprayed Bollgard II[®] plots (Jackson et al. 2005).

The findings in the present study illustrate the need for further examination of insecticide recommendations against target and non-target pests of Bollgard II[®] and other pyramided Bt cottons. Many current chemical control recommendations for fall armyworm on cotton do not differentiate between insecticide rates used on conventional and Bollgard II[®] fields (Akin et al. 2010, Andrews et al. 2010, Baldwin et al. 2010). The common practice of recommending

maximum rates of insecticides for fall armyworm management in Bollgard II® cultivars needs to be reconsidered. The results herein should be validated in a series of field trials to confirm satisfactory efficacy with lower rates. Future field and laboratory studies should evaluate insecticide performance (initial and residual efficacy) and dose-response on WideStrike™ and other pyramided Bt cotton traits. Finally, the fall armyworm provided an effective model insect for this study, but further research is needed to determine if similar results can be obtained when targeting other Lepidopteran pest populations, such as bollworm, in Bt cotton cultivars.

References Cited

- Adameczyk, J. J., Jr., J. Holloway, J. B. Graves, and B. R. Leonard. 1998.** Larval survival and development of the fall armyworm (Lepidoptera: Noctuidae) on normal and transgenic *Bacillus thuringiensis* (Bt) cotton. *J. Econ. Entomol.* 91: 539-545.
- Adameczyk, J. J., Jr., 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: 1589-1593.
- Adameczyk, J. J., Jr., S. Greenberg, J. S. Armstrong, W. J. Mullins, L. B. Braxton, R. B. Lassiter, and M. W. Siebert. 2008.** Evaluations of Bollgard®, Bollgard II®, and WideStrike® technologies against beet and fall armyworm larvae (Lepidoptera: Noctuidae). *Florida Entomol.* 91: 531-536.
- Akin, S., J. Carson, J. D. Hopkins, K. Loftin, G. Lorenz, P. Spradley, G. Studebaker, J. Zawislak, J. L. Bernhardt, D. T. Johnson, T. J. Kring, P. J. McLeod, M. V. Meisch, D. C. Steinkraus, and R. N. Weidenmann. 2010.** Cotton insect control, pp. 65-74. *In* G. Studebaker (ed.) MP144 2010 Insecticide Recommendations for Arkansas. University of Arkansas Division of Agriculture [Online]. Available at http://www.uaex.edu/Other_Areas/publications/PDF/MP144/MP-144.asp (verified 28 Mar. 2011).
- Ali, A., R. G. Luttrell, H. N. Pitre, and F. M. Davis. 1989.** Distribution of fall armyworm (Lepidoptera: Noctuidae) egg masses on cotton. *Environ. Entomol.* 18: 881-885.
- Ali, A., R. G. Luttrell, and H. N. Pitre. 1990.** Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. *Environ. Entomol.* 19: 1060-1067.
- Andrews, G., B. Burdine, A. Catchot, D. Cook, C. Daves, D. Dodds, J. Gore, R. Jackson, T. Koger, E. Larson, F. Musser, P. McKibben, J. Reed, J. Robbins, J. Smith, and S. Winters. 2010.** Cotton insect management, pp. 2-21. *In* A. Catchot (ed.) Insect Control Guide for Agronomic Crops 2010. Mississippi State University Extension Service

[Online]. Available at <http://msucares.com/pubs/publications/p2471.pdf> (verified 28 Mar. 2011).

Baldwin, J. L., L. D. Foil, M. L. Grodner, A. Hammond, G. Henderson, N. Hummel, S. Johnson, R. Leonard, A. Morgan, D. K. Pollet, J. Pyzner, T. E. Reagan, D. Reed, D. Ring, R. N. Story, and M. Stout. 2010. Louisiana recommendations for control on cotton insects, pp. 121-126. *In* 2010 Louisiana Insect Pest Management Guide. LSU AgCenter [Online]. Available at http://www.lsuagcenter.com/en/communications/publications/management_guides/Louisiana+Insect+Pest+Management+Guide.htm (verified 16 May 2011).

Bommireddy, P. L. and B. R. Leonard. 2008. Survivorship of *Helicoverpa zea* and *Heliothis virescens* on cotton plant structures expressing a *Bacillus thuringiensis* vegetative insecticidal protein. *J. Econ. Entomol.* 101: 1244-1252.

Chitkowski, R. L., S. G. Turnipseed, M. J. Sullivan, and W. C. Bridges, Jr. 2003. Field and laboratory evaluations of transgenic cottons expressing one or two *Bacillus thuringiensis* var. *kurstaki* Berliner proteins for management of Noctuid (Lepidoptera) pests. *J. Econ. Entomol.* 96: 755-762.

Cooper, S. G., D. S. Douches, and E. J. Grafius. 2006. Insecticidal activity of avidin combined with genetically engineered and traditional host plant resistance against Colorado potato beetle (Coleoptera: Chrysomelidae) larvae. *J. Econ. Entomol.* 99: 527-536.

Hardke, J. T., R. E. Jackson, B. R. Leonard, and J. H. Temple. 2011a. Fall armyworm (Lepidoptera: Noctuidae) development, survivorship, and damage on cotton plants expressing insecticidal plant-incorporated protectants. *J. Econ. Entomol.* (Accepted).

Hardke, J. T., J. H. Temple, B. R. Leonard, and R. E. Jackson. 2011b. Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). *Florida Entomol.* 94: 272-278.

IRAC International MoA Working Group. 2010. IRAC MoA classification scheme. Version 7.0 [Online]. Available at http://www.iraconline.org/wp-content/uploads/2009/09/MoA-classification_v7.0.4-5Oct10.pdf (verified Mar. 28, 2011).

Jackson, R. E., J. R. Bradley, Jr., and J. W. Van Duyn. 2003. Bollworm population production and associated damage in Bollgard and Bollgard II cottons under insecticide-treated and non-treated conditions, pp. 1022-1025. *In* Proc. Beltwide Cotton Conf., Nashville, TN. 6-10 Jan. 2003. Natl. Cotton Council Am., Memphis, TN.

Jackson, R. E., J. R. Bradley, and J. W. Van Duyn. 2005. Comparative efficacy of Bt technologies against bollworm in North Carolina, pp. 1373-1378. *In* Proc. Beltwide Cotton Conf., New Orleans, LA. 4-7 Jan. 2005. Natl. Cotton Council Am., Memphis, TN.

- Knipling, E. F. 1980.** Regional management of the fall armyworm—a realistic approach? Florida Entomol. 63: 468-480.
- Luginbill, P. A. 1928.** The fall armyworm. USDA Tech. Bull. 34. U.S. Gov. Print Office.
- Lynch, R. E., B. R. Wiseman, H. R. Sumner, D. Plaisted, and D. Warnick. 1999.** Management of corn earworm and fall armyworm (Lepidoptera: Noctuidae) injury on a sweet corn hybrid expressing a *cryIA(b)* gene. J. Econ. Entomol. 92: 1217-1222.
- Mink, J. S. and R. G. Luttrell. 1989.** Mortality of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs, larvae, and adults exposed to several insecticides on cotton. J. Entomol. Sci. 24: 563-571.
- Pitre, H. N. 1986.** Chemical control of the fall armyworm (Lepidoptera: Noctuidae): an update. Florida Entomol. 69: 570-578.
- Reed, J. T., and D. B. Smith. 2001.** Droplet size and spray volume effects on insecticide deposit and mortality of heliothine (Lepidoptera: Noctuidae) larvae in cotton. J. Econ. Entomol. 94: 640-647.
- SAS Institute. 2004.** SAS version 9.1 for Windows. SAS Institute, Cary, NC.
- Siebert, M. W., S. Nolting, B. R. Leonard, L. B. Braxton, J. N. All, J. W. Van Duyn, J. R. Bradley, J. Bacheler, and R. M. Huckaba. 2008.** Efficacy of transgenic cotton expressing Cry1Ac and Cry1F insecticidal protein against heliothines (Lepidoptera: Noctuidae). J. Econ. Entomol. 101: 1950-1959.
- Smith, J. F. and A. L. Catchot. 2009.** Efficacy of selected insecticides against fall armyworm on corn, 2008. Arthropod Management Tests 34: F18.
- Sparks, A. N. 1979.** A review of the biology of the fall armyworm. Florida Entomol. 62: 82-87.
- 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: 752-760.
- Tindall, K. V., B. R. Leonard, and K. Emfinger. 2006.** Evaluation of selected insecticides in a lab-field test, 2005. Arthropod Management Tests 31: F28.
- Williams, M. R. 2010.** Cotton insect losses – 2009, pp. 1029-1073. In Proc. Beltwide Cotton Conf., New Orleans, LA. 4-7 Jan. 2010. Natl. Cotton Counc. Am., Memphis, TN.
- Young, J. R. 1979.** Fall armyworm: control with insecticides. Florida Entomol. 62:130-133.
- Yu, S. J. 1983.** Age variation in insecticide susceptibility and detoxification capacity of fall armyworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 76: 219-222.

CHAPTER 6

FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (J. E. SMITH), IDENTIFICATION AND DAMAGE TO COTTON

Introduction

The fall armyworm is an occasional pest of Louisiana cotton, but has the potential to inflict serious damage when heavy infestations occur. In these situations, fall armyworms can be difficult to control with insecticide applications. In years when fall armyworm populations reach high levels, infestations of economic importance typically are scattered rather than dispersed across a broad production area. Most fall armyworm problems in Louisiana tend to occur in the northeast part of the state, where the majority of preferred host crops are grown. Light populations of fall armyworm have always been present in Louisiana, but in recent years population increases to damaging levels have become more frequent.

Species Description

Eggs. Fall armyworm eggs (Illustration 6.1) typically occur in an aggregate or mass and are found on the underside of leaves (abaxial surface) within the lower two-thirds of the cotton plant canopy (Ali et al. 1989). Number of eggs in a mass may be fewer than ten, but more often occur in clusters reaching several hundred. Egg masses are distinct in appearance, in that they are often found to be covered with a white down (Illustration 6.2) (Luginbill 1928). Eggs are typically greenish gray in color immediately after oviposition. Eggs darken with age, progressing from the initial greenish gray appearance to brown, and finally to nearly black just prior to larval eclosion (Illustration 6.3).



Illustration 6.1. Fall armyworm egg.



Illustration 6.2. Fall armyworm egg mass covered in scales (down).



Illustration 6.3. Fall armyworm egg mass with maturing eggs visible beneath layer of down.

Larvae. The fall armyworm is a caterpillar type of larva with four (4) pairs of abdominal prolegs. First instars are typically off-white to yellow in color with black head capsules (Illustration 6.4), which is similar in appearance to bollworm/tobacco budworm. Additional instars appear similar in color after molting (Illustration 6.5), but darken in color with age (Illustration 6.6). Later instars (Illustration 6.7) are dark in color, ranging from green to brown to nearly black, with head capsules varying from brown to black. The body is covered with black tubercles or bumps with stiff hairs, but it is less hairy than the bollworm (Illustration 6.8). Fall armyworm larvae possess a distinct inverted “Y” on the head capsule (Illustrations 6.9, 6.11) which becomes increasingly prominent with age. Additionally, on late instars, there are four prominent black spots in a square pattern on the dorsal surface of the last abdominal segments (Illustrations 6.10, 6.11). The dorsum, or back, of the caterpillar is marked by three longitudinal

stripes which are lighter in color than the main body. Other potential identifying marks may include a non-continuous white line in the mid-dorsal area, and/or intermittent yellow and red flecks on the abdomen (Oliver and Chapin 1981).



Illustration 6.4. Newly eclosed fall armyworm neonates.



Illustration 6.5. Early third-instar fall armyworm.



Illustration 6.6. Late third-instar fall armyworm.



Illustration 6.7. Late instar fall armyworm.

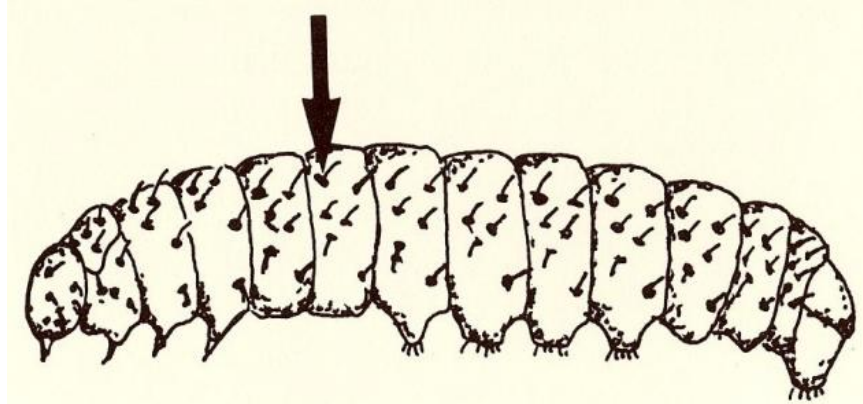


Illustration 6.8. Fall armyworm illustration showing hairs on the larva body (Adapted from Baldwin et al. 1990).



Illustration 6.9. Fall armyworm larva showing inverted "Y" on head capsule.



Illustration 6.10. Fall armyworm larva showing 4 prominent black dots in a square pattern on rear of abdomen.



Illustration 6.11. Fall armyworm larva feeding within a cotton boll showing inverted “Y” on head and 4 black dots in square pattern on end of abdomen.

Adults. Moths (Illustration 6.12) have a one and one-half inch wingspan. Forewing color varies from a mottled, dark gray at the top to a light gray or brown at the bottom (Illustration 6.13) (Oliver and Chapin 1981). A distinct white “spot” can be found near the tip of the wing. Hind wings are generally light gray to white in color. Male adults may be confused with yellow-striped armyworm, while female adults can be confused with beet armyworm. In addition, fall armyworm moths have filiform (threadlike) antennae and are generally active at night (nocturnal) (Sparks 1979).



Illustration 6.12. Fall armyworm moth at rest.



Illustration 6.13. Fall armyworm moth showing mottled brown forewings and tan hind wings.

Behavior and Damage

Fall armyworms are divided into two separate “strains” based on their host plant preference. The “rice-strain” prefers to feed on rice and turf grasses while the “corn-strain” prefers corn, cotton, and grain sorghum. It should be noted that the rice-strain can appear in cotton fields, depending on available plant hosts in the area. Some weed hosts can also be responsible for the appearance of rice-strain individuals in cotton.

Fall armyworm moths are nocturnal when they lay their eggs in masses. Moths usually deposit egg masses on the underside of leaves in the lower two-thirds of the plant canopy. Masses can range in size from as few as 10 eggs to as many as 500. Egg masses tend to be layered two to five eggs deep. In general appearance, egg masses are dime-sized and exhibit a fuzzy gray appearance caused by a covering of scales from the female moth. Female moths can

lay approximately 150 eggs a day for eight to 10 days. Once deposited on plants, eggs hatch in two to four days depending on environmental conditions.

Damage in cotton fields from fall armyworm outbreaks can be unpredictable, and include feeding injury to a range of plant structures during vegetative and reproductive stages of development. Neonates and early instars skeletonize leaves by feeding only on the lower leaf surface, also known as a “windowpane” effect (Illustration 6.14). As larvae disperse from the site of the egg mass, some larvae produce silk-like threads which they use to descend to lower areas of the plant. As larvae increase in size, they may disperse to neighboring plants.



Illustration 6.14. Fall armyworm feeding on leaf tissue by young larvae.

While small fall armyworms prefer to feed on cotton foliage or grasses, if present in the field, large worms attack cotton fruiting structures. Later instars migrate from the site of eclosion and may infest a variety of plant structures. These stages will directly injure reproductive structures such as squares (flower buds), blooms (flowers), and bolls (capsules) (Illustrations 6.15A, B, and C) (Ali et al. 1990). On vegetative stage plants or on plants in the absence of fruiting forms, late instars have the potential to destroy terminal buds and cause loss

of apical dominance (Leigh et al. 1996). On reproductive stage plants with fruiting forms, later instars prefer to feed on bracts (Illustrations 6.16A, B), large squares, blooms, and young bolls. Heavy infestations may damage all fruiting structures. Late instars also have the ability to bore into and feed within fruiting structures (Illustration 6.17). As larvae develop, it is more common to find injury on blooms and older bolls lower in the plant canopy, but they may occasionally be seen feeding on squares. Damaged fruiting forms can be difficult to identify, as larvae prefer to enter these structures from the base near the bracts and in some cases only surface feeding (etching) is evident (Illustration 6.19). Based upon field observations, the fall armyworm damages fewer fruiting forms than bollworm, but is capable of attacking and damaging bolls no longer susceptible to bollworms. The larval stage lasts about three weeks, at the end of which fall armyworms move to the ground and pupate in the soil (Illustration 6.18).



Illustration 6.15A. Fall armyworm damage to cotton square.



Illustration 6.15B. Fall armyworm damage to cotton bloom and young boll.



Illustration 6.15C. Fall armyworm damage to cotton boll.



Illustration 6.16A. Fall armyworm feeding damage on square and square bracts.



Illustration 6.16B. Fall armyworm feeding on boll bracts.



Illustration 6.17. Fall armyworm feeding inside a cotton boll.



Illustration 6.18. Fall armyworm pupa.

It is possible to find fall armyworms in cotton throughout the growing season, but heavy infestations typically occur later in the year (August and September). At this time, preferred hosts such as corn and grain sorghum have reached maturity and are no longer attractive or viable hosts. Dry years also seem to be associated with increases in fall armyworm problems, which is likely a result of dry pastures and grasslands not providing alternate hosts. Cotton fields

that are irrigated and/or planted late may be more attractive late in the season compared to dry or cut-out (mature) fields.

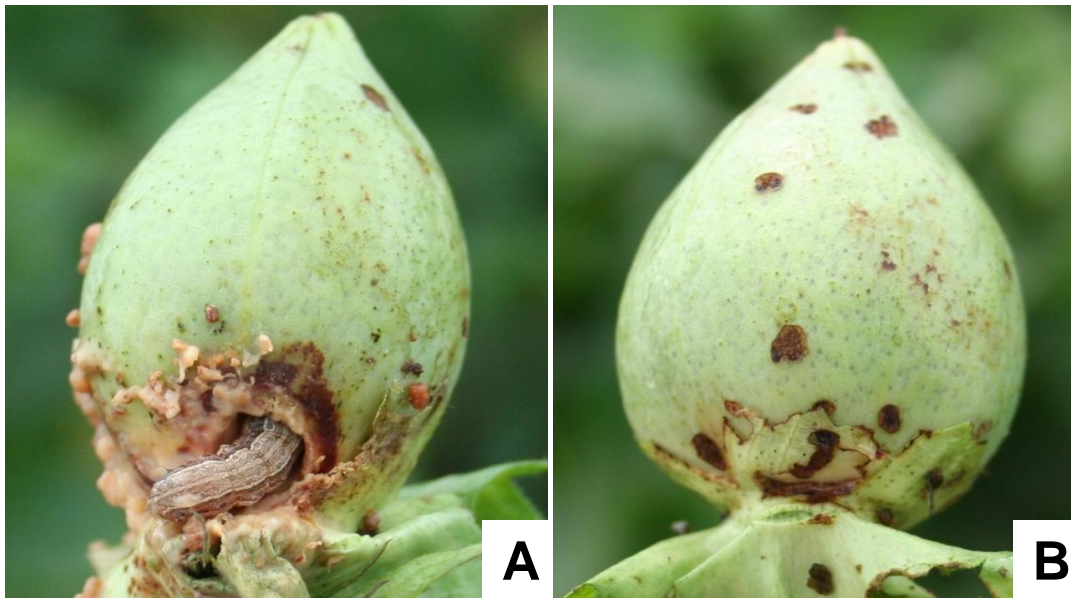


Illustration 6.19. Fall armyworm damage – complete penetration (A) compared to “etching” (B).

Scouting

Typical bollworm scouting methods are not effective for monitoring fall armyworm infestations. Fall armyworms do not lay their eggs in the mainstem terminal area, and small larvae do not consistently feed on squares. Due to this behavior, standard terminal and square sampling techniques are not reliable.

It is difficult to properly scout for small fall armyworms because their behavior can be unpredictable at times. Whole plant evaluations, though time-consuming, are the most reliable means of scouting for fall armyworms. In contrast, large fall armyworms can be found feeding on blooms and bolls in the lower two-thirds of the plant canopy (Illustration 6.20). Fall armyworms will feed on bracts of bolls and squares, which is uncharacteristic of bollworms. However, excessive defoliation is not a common symptom of fall armyworm infestations in cotton.

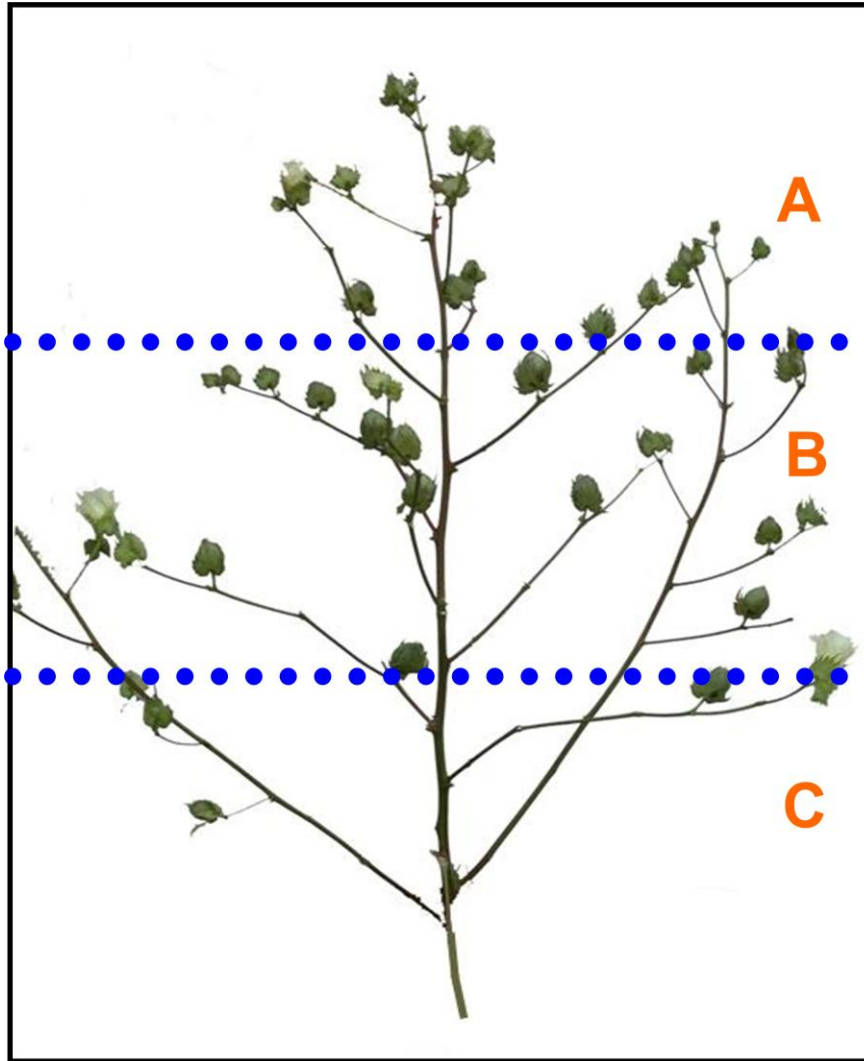


Illustration 6.20. Fall armyworm egg masses are typically found in the lower two-thirds of the plant canopy.

Monitoring for egg masses is a potentially effective means of detecting fall armyworm infestations. Scouting for egg masses involves bending over random plants and examining the underside of leaves (Illustration 6.21). Egg masses may potentially be found anywhere on the plant, particularly during periods of heavy egg lay, but eggs are predominately laid on the bottom of leaves. Given the difficulty in detecting small fall armyworms, scouting for egg masses is more effective and prevent infestations from going undetected until large larvae are present and feeding on fruiting structures.



Illustration 6.21. Newly hatched fall armyworm larvae on the under surface of a leaf.

Fall armyworm infestations in cotton can occasionally be associated with fields where weed control measures have been inadequate. Fall armyworms of the rice-strain may feed on grasses in and/or on the edge of the field before moving onto cotton once the grass host is no longer appealing. However, the absence of weeds in cotton fields does not lessen the potential for fall armyworm infestations.

The current action threshold for fall armyworms is to treat when egg masses or small larvae appear. Scouting emphasis should be placed on detecting egg masses and then spraying for worms immediately upon hatching.

Control

Insecticides. Fall armyworms can be difficult to control based upon their dispersal in the lower two-thirds of the plant canopy. Insecticide deposition is inadequate in these areas and can lead to control failures. When cotton plants are tall with a dense canopy, the lower areas of the plant where fall armyworms occur receive less insecticide.

Current insecticide control recommendations for fall armyworm include a variety of chemistries. Pyrethroids, such as Baythroid XL, Karate Z, Mustang Max, and Prolex (Table 6.1), may be effective in controlling fall armyworms if applied at high rates and targeted against small larvae. In situations where large fall armyworms are being treated, Belt, Coragen, Diamond*, Intrepid, Larvin, and Tracer may be used to provide adequate control. In situations where large larvae have already attacked bolls, these materials may not be cost effective. For that reason, early detection of fall armyworm infestations is important to control this pest in an economical manner. The 2010 Louisiana Pest Management Guide can be found on-line:

<http://www.lsuagcenter.com/NR/rdonlyres/B733BE31-7DEA-4264-93A6-AF6FED7023D3/66760/pub1838InsectPestMgmtGuide2010completebook188pgs.pdf>.

*Diamond should be tank mixed with another recommended insecticide.

Bt Cotton. The first Bt cotton, Bollgard[®], was designed to express the Cry1Ac toxin to control the tobacco budworm, against which it remains very effective. Bollgard II[®] and WideStrike[™] cottons, expressing multiple Bt toxins, have increased the spectrum of lepidopteran pest control. Fall armyworms are not susceptible to Bollgard[®] cotton and supplemental sprays may be needed in order to control infestations (Illustration 6.22). Bollgard[®] II can effectively suppress fall armyworms under low infestation levels. WideStrike[™] generally provides sufficient control of fall armyworms except under extreme infestation levels. Evidence of surface feeding is not uncommon in Bt cotton fields (Illustration 6.23), and it is important to note that Bt cottons are not immune to injury from fall armyworm (Illustration 6.24). Depending upon the severity of the infestation, all of these cottons may require supplemental insecticide treatment to control fall armyworms.



Illustration 6.22. Fall armyworm penetrating a non-Bt or Bollgard[®] cotton boll.



Illustration 6.23. Fall armyworm etching on a Bt cotton boll.



Illustration 6.24. Deceased fall armyworm larva having successfully penetrated a Bt cotton boll.

The authors wish to acknowledge that numerous references contributed to the development of this chapter, including, but not limited to, Ali et al. 1989, Ali et al. 1990, Leigh et al. 1996, Luginbill 1928, Oliver and Chapin 1981, and Sparks 1979.

References Cited

- Ali, A., R. G. Luttrell, H. N. Pitre, and F. M. Davis. 1989.** Distribution of fall armyworm (Lepidoptera: Noctuidae) egg masses on cotton. *Environ. Entomol.* 18: 881-885.
- Ali, A., R. G. Luttrell, and H. N. Pitre. 1990.** Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. *Environ. Entomol.* 19: 1060-1067.
- Baldwin, J. L., J. B. Graves, and R. Leonard. 1990.** Armyworms in cotton. Louisiana Coop. Ext. Service. Pub. 2377.
- Baldwin, J. L., L. D. Foil, M. L. Grodner, A. Hammond, G. Henderson, N. Hummel, S. Johnson, R. Leonard, A. Morgan, D. K. Pollet, J. Pyzner, T. E. Reagan, D. Reed, D.**

- Ring, R. N. Story, and M. Stout. 2010.** Louisiana recommendations for control on cotton insects, pp. 121-126. *In* 2010 Louisiana Insect Pest Management Guide. LSU AgCenter [Online]. Available at http://www.lsuagcenter.com/en/communications/publications/management_guides/Louisiana+Insect+Pest+Management+Guide.htm (verified 16 May 2011).
- Leigh, T. F., S. H. Roach, and T. F. Watson. 1996.** Biology and ecology of important insect and mite pests of cotton, pp. 17-85. *In* E. G. King, J. R. Phillips, and R. J. Coleman (eds.) Cotton insects and mites: characterization and management. (No. 3) The Cotton Foundation Reference Book Series.
- Luginbill, P. 1928.** The fall armyworm. USDA Tech. Bull. No. 34.
- Oliver, A. D. and J. B. Chapin. 1981.** Biology and illustrated key for the identification of twenty species of economically important Noctuid pests. Louisiana Agric. Expt. Stn. Bull. No. 733.
- Sparks, A. N. 1979.** A review of the biology of the fall armyworm. Florida Entomol. 62: 82-87.

Table 6.1. Fall Armyworm Chemical Control Options (Baldwin et al. 2010).

Insecticide Trade Name	Insecticide Common Name	Product Formulation	Amount Product Per Acre	lb Active Ingredient Per Acre	Acres Treated Per Gallon or Pound
Baythroid XL	cyfluthrin	1.0 lb AI/gal	3.2 oz	0.025	40
	cyfluthrin	2.0 lb AI/gal	2.6 oz	0.041	50
Belt	flubendiamide	4.0 lb AI/gal	2 – 3 oz	0.063 – 0.094	64 – 42.7
Coragen	chlorantraniliprole	1.67 lb AI/gal	2 – 4 oz	0.044 – 0.088	8 – 4
Diamond	novaluron	0.83 lb AI/gal	6 – 12 oz	0.039 – 0.077	21.3 – 10.6
Intrepid	methoxyfenozide	2 lb AI/gal	6 – 10 oz	0.09 – 0.16	21 – 12.5
Karate Z	lambda-cyhalothrin	2.08 lb AI/gal	2.56 oz	0.04	50
Larvin	thiodicarb	3.2 lb AI/gal	36 oz	0.9	3.5
Mustang Max	zeta-cypermethrin	0.8 lb AI/gal	4 oz	0.025	32
Declare	gamma-cyhalothrin	1.25 lb AI/gal	2.05 oz	0.02	62.5
Tracer	spinosad	4 lb AI/gal	2.8 oz	0.089	45

CHAPTER 7

SUMMARY AND CONCLUSIONS

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is an occasional pest of cotton across the United States. This insect has traditionally been controlled with conventional chemical control strategies of insecticides. During the last decade, cotton plants expressing *Bacillus thuringiensis* (Bt) proteins have emerged as the primary tool for managing many lepidopteran pests in cotton arthropod pest management systems. The first cotton line to express a Bt protein (Cry1Ac) was commercialized as the Bollgard[®] trait and was highly effective against the tobacco budworm, *Heliothis virescens* (F.), pink bollworm, *Pectinophora gossypiella* (Saunders), and European corn borer, *Ostrinia nubilalis* (Hübner). Unfortunately, the target insect spectrum for this technology was limited, which still necessitated foliar applications of insecticides to control additional lepidopteran pests. Bollgard II[®] (Cry1Ac + Cry2Ab) and WideStrike[™] (Cry1Ac + Cry1F) were subsequently commercialized and express pyramided Bt proteins. Both of these traits have broadened the spectrum of control to include additional lepidopteran species beyond that controlled by the single protein expressed in Bollgard[®]. In addition, new insecticides with novel modes of action have been developed in recent years that demonstrate better efficacy against lepidopteran pests compared to that of traditional insecticides. These newer compounds have the potential to significantly improve cotton insect pest management. Currently, limited work has been completed that characterizes the effects of Bt cotton technologies and many of the new insecticides on fall armyworm. Therefore, the objective of these studies was to evaluate fall armyworm larval survivorship and plant injury on Bt and non-Bt cotton, adult oviposition behavior on Bt cotton lines compared to non-Bt cotton lines, and fall armyworm susceptibility to insecticides in Bt and non-Bt cotton.

In our research, field and laboratory studies quantified fall armyworm survivorship, development, and damage on cotton fruiting forms (squares, white flowers, and bolls) of selected Bt cotton lines (Bollgard[®], Bollgard II[®], and WideStrike[™]) compared to a non-Bt cotton line. Significant differences were detected between Bt and non-Bt cottons in their effectiveness against fall armyworm. The single protein, Cry1Ac, expressed in Bollgard[®] cotton fruiting forms was generally ineffective in reducing fall armyworm survivorship or preventing damage to cotton fruiting forms compared to results for non-Bt cotton lines in field and laboratory studies. Fall armyworm survivorship and damage was >40% and >61%, respectively, across all Bollgard[®] fruiting forms in field studies. Results of the laboratory studies supported field observations and showed that Bollgard[®] did not affect fall armyworm development on squares and white flowers. However, continuous exposure to bolls from Bollgard[®] plants significantly increased pupal duration and reduced percentages of larvae surviving to adults. The pyramided cotton trait (Cry2Ab to Cry1Ac), Bollgard II[®], produced effects on fall armyworm that were not as consistent as those observed for those on Bollgard[®] plants for field and laboratory tests. Field studies showed minimal effects on fall armyworm from feeding on Bollgard II[®] tissues, with survivorship and damage of >25% and >45%, respectively, across all fruiting forms. Laboratory studies indicated a reduction in fall armyworm survivorship on all Bollgard II[®] fruiting forms and a decrease in injury to squares, but not to bolls. The inclusion of the additional protein in Bollgard II[®] cotton lines appeared to improve efficacy against fall armyworm, but the cumulative effects were inconsistent. These results suggest that the expression of Bt proteins is influenced by plant tissues and that the effective doses are very close to the critical levels required to negatively affect fall armyworm. Cotton plants expressing the WideStrike[™] (Cry1F and Cry1Ac) trait consistently reduced fall armyworm survivorship (>14%) and subsequent injury

(>19%) across all fruiting forms in field studies. Similar reductions in survivorship and injury were observed in laboratory experiments. These effects with WideStrike™ could indicate a more active pyramid of products or higher expression of one or both proteins against fall armyworm.

Fall armyworm oviposition behavior was determined on a non-Bt cotton line and compared with that on Bollgard®, Bollgard II®, and WideStrike™ cotton lines. None of the Bt cotton lines caused a significant change in the frequency (1.44 to 2.33 egg masses recovered per infestation event) or distribution of egg masses (8.7 to 11.1 average main stem node) on cotton plants. Fall armyworm adult behavior associated with oviposition on cotton plants is not affected by the proteins expressed in current commercially-available Bt cotton lines. Cotton pest managers should not need to change from any of the currently recommended field sampling protocols and continue to use a single method to evaluate fall armyworm infestations in both Bt and non-Bt cotton fields.

The contact and residual efficacy of selected insecticides was determined in laboratory studies using insecticide-treated Bt and non-Bt cotton tissue allowed to weather in a field environment. Insecticides (chlorantraniliprole, flubendiamide, and spinetoram) with novel modes of action generally produced greater fall armyworm mortality than the commercial standard insecticides (lambda-cyhalothrin and novaluron) on both Bollgard II® and non-Bt cotton. Reduced rates of these insecticides on Bollgard II® cotton terminal leaves and bolls produced fall armyworm mortality equivalent to that observed for full rates of each respective product on non-Bt cotton plant structures. Opportunities to reduce insecticide rates without compromising satisfactory control allows the cotton industry to reduce chemical control costs and provide more environmentally-sustainable management options.

Limited information has been developed, summarized, or published in stakeholder-friendly venues during the previous decade on identification and characterization of injury to cotton for fall armyworm. Through other objectives, a comprehensive report providing keys for pest identification, field ecology, and injury to cotton was generated in an electronic format. The information for this report has been formatted in a manner that will be useful to cotton pest managers and producers to access and improve references for the decision-making process of cotton integrated pest management.

The conclusions of this project substantially add to the understanding of fall armyworm biology and ecology in cotton, provide a basis for managing this insect in Bt cotton lines, demonstrate efficacy of novel chemical control strategies, and offer easy-to-access reference information on fall armyworm in cotton. Results indicate that none of the Bt cotton lines currently available are immune to fall armyworm damage, and in some instances larvae were capable of completing development to adulthood. Therefore, supplemental insecticide applications may be needed to manage severe fall armyworm infestations even in pyramided Bt cotton fields. Fall armyworm adult oviposition was similar between both Bt and non-Bt cotton lines. Cotton pest managers should therefore be justified in using a single scouting procedure for detecting fall armyworm eggs and small larvae in both Bt and non-Bt cotton fields. The effectiveness of new insecticides against late-stage fall armyworm larvae offers options to achieve satisfactory control of this pest in both Bt and non-Bt cotton fields. However, further research is needed to confirm the effectiveness of reduced rates of insecticides in pyramided Bt cotton fields. This information, combined with written and visual descriptions of fall armyworm and its damage to cotton, should aid in the improvement of fall armyworm management with Bt cotton and insecticides.

This project has addressed several critical issues with fall armyworm management in cotton, but opportunities for future research with this pest and its relationship to cotton should be considered. The general areas of research include:

- 1) Laboratory studies are needed to determine the progression of fall armyworm feeding preference over time (i.e. first instars prefer lower canopy leaves, second instars move to feed on small squares, etc.) on non-Bt and Bt cotton lines (more specifically, determine fall armyworm preference for various cotton tissues on non-Bt and Bt lines at different insect growth stages),
- 2) Field studies are needed to determine fall armyworm preference for egg deposition on non-Bt versus Bt cotton plants and possible implications for management of this pest across a farmscape,
- 3) Laboratory studies are needed to determine the effects of consumption of Bt cotton tissue on fall armyworm fecundity and the subsequent impact on future generations,
- 4) Field tests are needed to confirm the effectiveness of reduced rates of insecticides in pyramided Bt cotton fields, and finally
- 5) Laboratory tests are needed to determine the damage and survivorship of Cry1F-resistant fall armyworm from Puerto Rico on pyramided Bt cotton lines.

APPENDIX

LETTER OF PERMISSION FOR CHAPTER 3

Letter of permission from the Entomological Society of America's Journal of Economic Entomology to reprint Chapter 3.

RE: JEE Manuscript EC-11-108

Alan Kahan [akahan@entsoc.org]

Sent: Tuesday, May 17, 2011 7:48 AM

To: Hardke, Jarrod

May 16, 2011

Jarrod T. Hardke
Row Crop IPM
LSU AgCenter
Dept. of Entomology

Dear Jarrod Hardke,

The Entomological Society of America grants you permission to reprint the data from the article cited below as a chapter of your dissertation for Louisiana State University.

Hardke, Jarrod T. 2011. Fall armyworm (Lepidoptera: Noctuidae) development, survivorship, and damage on cotton plants expressing insecticidal plant-incorporated protectants. *Journal of Economic Entomology*. 104: (in press)

Please provide proper acknowledgement.

Best wishes,

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VITA

Jarrod Tyler Hardke is the son of Gary and Melodie Hardke. He was born in December 1983, in Carlisle, Arkansas. He graduated from Carlisle High School in May 2002. He earned a Bachelor of Science in Agricultural, Food and Life Sciences (crop management and pest management majors) from the University of Arkansas in December 2006. In January 2007, he began his graduate studies under the direction of Dr. B. Rogers Leonard studying insect pest management in Louisiana field crops. Jarrod is currently a doctoral candidate in the Department of Entomology at Louisiana State University and Agricultural and Mechanical College.