Lepidoptera (Crambidae, Noctuidae, and Pyralidae) Injury to Corn Containing Single and Pyramided Bt Traits, and Blended or Block Refuge, in the Southern United States

D. D. REISIG,[1] D. S. AKIN,[2] J. N. ALL,[3] R. T. BESSIN,[4] M. J. BREWER,[5] D. G. BUNTIN,[6]
A. L. CATCHOT,[7] D. COOK,[8] K. L. FLANDERS,[9] F.-N. HUANG,[10] D. W. JOHNSON,[11]
B. R. LEONARD,[12] P. J. MCLEOD,[13] R. P. PORTER,[14] F.P.F. REAY-JONES,[15] K. V. TINDALL,[16]
S. D. STEWART,[17] N. N. TROXCLAIR,[18] R. R. YOUNGMAN,[19] AND M. E. RICE[20,21]

J. Econ. Entomol. 108(1): 157–165 (2015); DOI: 10.1093/jee/tou009

ABSTRACT Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae); corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae); southwestern corn borer, *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae); sugarcane borer, *Diatraea saccharalis* F. (Lepidoptera: Crambidae); and lesser cornstalk borer, *Elasmopalpus lignosellus* Zeller (Lepidoptera: Pyralidae), are lepidopteran pests of corn, *Zea mays* L., in the southern United States. Blended refuge for transgenic plants expressing the insecticidal protein derivative from *Bacillus thuringiensis* (Bt) has recently been approved as an alternative resistance management strategy in the northern United States. We conducted a two-year study with 39 experiments across 12 states in the southern United States to evaluate plant injury from these five species of Lepidoptera to corn expressing Cry1F and Cry1Ab, as both single and pyramided traits, a pyramid of Cry1Ab × Vip3Aa20, and a pyramid of Cry1F × Cry1Ab plus non-Bt in a blended refuge. Leaf injury and kernel damage from corn earworm and fall armyworm, and stalking tunneling by southwestern corn borer, were similar in Cry1F × Cry1Ab plants compared with the Cry1F × Cry1Ab plus non-Bt blended refuge averaged across five-plant clusters. When measured on an individual plant basis, leaf injury, kernel damage, stalk tunneling (southwestern corn borer), and dead or injured plants (lesser cornstalk borer) were greater in the blended non-Bt refuge plants compared to Cry1F × Cry1Ab plants in the non-Bt and pyramided Cry1F × Cry1Ab blended refuge treatment. When non-Bt blended refuge plants were compared to a structured refuge of non-Bt plants, no significant difference was detected in leaf injury, kernel damage, or stalk tunneling (southwestern corn borer). Plant stands in the non-Bt and pyramided Cry1F × Cry1Ab blended refuge treatment had more stalk tunneling from sugarcane borer and plant death from lesser cornstalk borer compared to a pyramided Cry1F × Cry1Ab structured refuge treatment. Hybrid plants containing Cry1F × Cry1Ab within the pyramided Cry1F × Cry1Ab blended refuge treatment had significantly less kernel damage than non-Bt structured refuge treatments. Both single and pyramided Bt traits were effective against southwestern corn borer, sugarcane borer, and lesser cornstalk borer.

KEY WORDS blended refuge, structured refuge, *Helicoverpa zea*, *Spodoptera frugiperda*

---

[1] Department of Entomology, North Carolina State University, Vernon G. James Research and Extension Center, 207 Research Station Rd., Plymouth, NC 27962.

[2] University Arkansas, Cooperative Extension Service, Monticello, AR 71656; currently, FMC Corporation, 1672 Hwy 138, Monticello, AR 71655.

[3] Department of Entomology, University of Georgia, Athens, GA 30602.

[4] Department of Entomology, S-225 Ag North, Lexington, KY 40546.

[5] Texas A&M AgriLife Research & Extension Center, 10345 State Hwy 44, Corpus Christi, TX 78406.

[6] Department of Entomology, UGA-Griffin Campus, 1109 Experiment Street, Griffin, GA 30223.

[7] Department of Entomology & Plant Pathology, Mississippi State University, Mississippi State, MS 39762

[8] Delta Research & Extension Center, Mississippi State University, P.O. Box 197, Stoneville, MS 38776.

[9] 201 Extension Hall, Auburn University, AL 36849.

[10] Department of Entomology, 404 Life Sciences Bldg., LSU AgCenter, Baton Rouge, LA 70803.

[11] UK-REC, 1205 Hopkinsville Street, Princeton, KY 42445.

[12] LSU AgCenter, Macon Ridge Station, 212A Macon Ridge Rd., Winnsboro, LA 71295.

[13] Cralley-Warren Lab, 2601 N. Young Ave., Fayetteville, AR 72704.

[14] Texas AgriLife Research & Extension Center, Lubbock, TX 79403.

[15] School of Agricultural, Forest, and Environmental Sciences, Pee Dee Research & Education Center, Clemson University, 2200 Pocket Rd., Florence, SC 29506.

[16] Division of Plant Sciences, University of Missouri, Portageville, MO 63873.

[17] West Tennessee Research and Education Center, 605 Airways Blvd., Jackson, TN 38301.

[18] Texas AgriLife Research & Extension Center, P.O. Box 1849, Uvalde, TX 78802.

[19] Department of Entomology, 216-A Price Hall, MC 0319, Virginia Tech University, Blacksburg, VA 24061.

[20] DuPont Pioneer, P. O. Box 1004, Johnston, IA 50131.

[21] Corresponding author, e-mail: marlin.rice@pioneer.com.

Introduction

Transgenic corn, *Zea mays* (L.) expressing the insecticidal protein derivative from *Bacillus thuringiensis* (Bt) Berliner is an important part of pest management in the United States. In 2013, ~76% of corn planted within the U.S. Corn Belt was a Bt-expressing hybrid (NASS 2013). While the European corn borer (Hübner) (Lepidoptera: Crambidae) has greatly diminished as a keystone pest in the U.S. Corn Belt (Hutchison et al. 2010), other lepidopterans, such as fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Noctuidae), and corn earworm, *Helicoverpa zea* (Boddie) (Noctuidae), persist as occasional pests in southern U.S. corn production (Buntin et al. 2004). Fall armyworm and corn earworm can injure corn by feeding on unfurled leaves in the whorl or on developing kernels in the ear; occasionally, fall armyworm tunnel into the cornstalk and ear shanks. Other lepidopteran corn pests in the southern United States include southwestern corn borer, *Diatraea grandiosella* Dyar (Crambidae); sugarcane borer, *Diatraea saccharalis* (F.) (Crambidae); and lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (Pyralidae). Both southwestern corn borer and sugarcane borer feed within the whorl during early larval stages and occasionally tunnel the corn stalk during later stages (Davis et al. 1972, Hensley 1971). The lesser cornstalk borer can injure seedling corn by feeding at or just below the soil surface, which can damage the growing point and subsequently kill the young plant.

Corn hybrids expressing insecticidal Bt proteins are widely deployed against these lepidopteran pests throughout the southern United States (Siebert et al. 2012). Single-gene Bt corn hybrids, which include event Bt 11 (Cry1Ab gene, Syngenta Seeds, Raleigh, NC) or event MON810 (Cry1Ab gene, Monsanto Co., St. Louis, MO), were originally deployed for the European corn borer; these hybrids can also be effective against other pests. For example, fall armyworm and corn earworm-induced whorl and ear damage can be reduced by >90% and 50–80%, respectively, in corn hybrids containing these transgenic events (Storer et al. 2001, Buntin et al. 2004).

Pyramiding Bt toxins is a more recent strategy to increase efficacy against some pests and delay insect resistance. Seed companies introduced commercial hybrids with pyramided transgenes in 2008 (Onstad et al. 2011). Pyramided genes can have greater efficacy and range of pest spectrum controlled (Burkness et al. 2010, Storer et al. 2010b), and have been suggested as a superior strategy to delay resistance (Bates et al. 2005). Pyramided Bt toxins in corn with efficacy against fall armyworm and corn earworm currently include Cry1Ab × Vip3Aa20, Cry1A.105 × Cry2Ab2, Cry1Ab × Cry1F, and Cry1A.105 × Cry2Ab2 × Cry1F (Flanders 2013). Depending on the specific transgene pyramid, such corn hybrids can be effective against a wide range of southern lepidopteran pests such as corn earworm, fall armyworm, southwestern corn borer, sugarcane borer, lesser cornstalk borer, and beet armyworm, *Spodoptera exigua* (Hübner) (Noctuidae) (Siebert et al. 2012).

In addition to delaying insect resistance by using pyramided Bt corn, a blended refuge, also known as seed mixture, integrated refuge, or refuge-in-the-bag, has been suggested as an improved method of resistance management that could help offset refuge noncompliance by growers (Gould 1986), compared to a structured refuge, although Burkness et al. (2011) counter that a blended refuge may undermine a refuge resistance management strategy. One survey estimated that U.S. corn grower refuge compliance was 92% from 2003 to 2005, but had dropped to 66–78% by 2008 (Jaffe 2009). However, in cotton (*Gossypium hirsutum* L.) regions of the southern United States, only 41% of surveyed Bt corn growers met the refuge size requirements and 32% planted no refuge (ABSTC 2013). Therefore, a lack of refuge compliance could contribute to the development of resistance and a blended refuge would possibly provide a solution to improving the durability of Bt traits. Blended refuge products were first approved as a supplemental resistance management strategy beginning in 2010 with corn hybrids containing the pyramided Bt genes Cry1Ab × Cry1F. Blended-refuge corn products were not approved for planting in cotton-growing counties in the southern United States without an additional structured block refuge for this product.

There are no published studies to date on the field efficacy of corn hybrids with pyramided insecticidal proteins using a blended refuge against southern U.S. lepidopteran pests. In corn, the effectiveness of the blended-refuge strategy for insect resistance management may be compromised depending on the pest species by larval movement among non-Bt refuge plants and those plants expressing Bt proteins (Bates et al. 2005). Additionally, larval mortality can increase in refuge plants when the kernels are cross-pollinated by surrounding Bt-expressing plants (Chilcutt and Tabashnik 2004). Conversely, mortality of ear-feeding larvae can also be reduced on plants expressing Bt due to cross-pollination (Burkness et al. 2011).

There were two main objectives in this study, to compare injury between several single and pyramided Bt traits and to compare injury between a blended refuge and a structured refuge. We evaluated plant injury to corn containing both single traits (Cry1Ab and Cry1F) and pyramided traits (Cry1F × Cry1Ab and Cry1Ab × Vip3Aa20), as well as the pyramided Cry1F × Cry1Ab plus non-Bt in a blended refuge against several lepidopteran pests in the southern United States. Emphasis was placed on the fall armyworm and corn earworm, but opportunistic data also were gathered on southwestern corn borer, sugarcane borer, and lesser cornstalk borer.

Materials and Methods

Experimental Locations and Design. A two-year study (2010–2011) evaluated seven treatments using small plots in separate tests across 12 southern states (Table 1).

Table 1. Location, planting date, and data collected for trials evaluating efficacy of Bt corn deployed against lepidopteran pests in 39 locations across 12 states in the southern United States

Location (county, state)	Year	Planting date	Data collected
Lee, AR	2010	13 May	Leaf injury, kernel area consumed, tunnel length (southwestern corn borer)
Desha, AR	2010	13 May	Leaf injury, kernel area consumed
Burke, GA	2010	21 July	Percent dead or injured plants (lesser cornstalk borer)
Burke, GA	2010	15 August	Percent dead or injured plants (lesser cornstalk borer)
Pike, GA	2010	12 May	Leaf injury, kernel area consumed
Caldwell, KY	2010	26 May	Kernel area consumed, tunnel length (southwestern corn borer)
Lexington, KY	2010	28 May	Leaf injury[a], kernel area consumed
Washington, NC	2010	24 May	Leaf injury, kernel area consumed
Pemiscot, MO	2010	25 May	Kernel area consumed, tunnel length (southwestern corn borer)
Uvalde, TX	2010	5 August	Leaf injury, kernel area consumed
Baldwin, AL	2010	6 May	Leaf injury, kernel area consumed
Franklin, LA	2010	18 May	Leaf injury, kernel area consumed, tunnel length (sugarcane borer)
Lubbock, TX	2010	9 June	Leaf injury, kernel area consumed
Florence, SC	2010	11 May	Leaf injury, kernel area consumed
Jackson, TN	2010	24 May	Kernel area consumed[b,c], tunnel length (southwestern corn borer)[b,c]
Blacksburg, VA	2010	Late May	Leaf injury, kernel area consumed
Corpus Christi, TX	2010	4 May	Kernel area consumed
Stoneville, MS	2010	14 May	Leaf injury[b], tunnel length (southwestern corn borer)[b]
Alexandria, LA	2010	27 May	Leaf injury, kernel area consumed, tunnel length (sugarcane borer)[d]
Burke, GA	2011	26 July	Percent dead or injured plants (lesser cornstalk borer)
Burke, GA	2011	6 September	Percent dead or injured plants (lesser cornstalk borer)
Pike, GA	2011	12 May	Leaf injury, kernel area consumed
Montgomery, VA	2011	1 June	Leaf injury, kernel area consumed
Stoneville, MS	2011	10 May	Kernel area consumed[b], tunnel length (southwestern corn borer)[b]
Caldwell, KY	2011	2 June	Leaf injury, kernel area consumed
Fayette, KY	2011	31 May	Leaf injury[a], tunnel length (southwestern corn borer)
Crawford, AR	2011	17 May	Leaf injury
Desha, AR	2011	23 May	Leaf injury, kernel area consumed
Alexandria, LA	2011	11 May	Kernel area consumed, tunnel length (sugarcane borer)
Hale, TX	2011	26 May	Leaf injury, kernel area consumed
Baldwin, AL	2011	3 May	Leaf injury, kernel area consumed
Florence, SC	2011	27 April	Kernel area consumed
Pemiscot, MO	2011	11 May	Kernel area consumed[c], tunnel length (southwestern corn borer)[c]
Corpus Christi, TX	2011	8 Sept.	Leaf injury, kernel area consumed
Jackson, TN	2011	9 May	Kernel area consumed[c,d], tunnel length (southwestern corn borer)[c,e]
Jackson, TN	2011	31 May	Kernel area consumed[c,d], tunnel length (southwestern corn borer)[c,e]
Winnsboro, LA	2011	10 May	Leaf injury, kernel area consumed
Oktibbeha, MS	2011	3 June	Leaf injury
Washington, NC	2011	25 May	Leaf injury, kernel area consumed

[a] Leaf injury resulted from artificial infestation of fall armyworm on 26 June 2010 or 1 July 2011. All other data regarding insect injury resulted from natural infestations.

[b] 20 plants or ears analyzed per plot.

[c] Refuge (non-Bt) plants in blended refuge treatment not included in analyses.

[d] Stalk tunnels resulted from artificial infestation of five neonates of sugarcane borer per plant at VT-R1 plant stages.

[e] Five plants or ears analyzed per plot.

Treatments were corn hybrids containing:

1) Cry1F × Cry1Ab pyramid of TC1507 × MON810 × NK603 (event TC1507, Cry1F protein, Dow AgroSciences, Indianapolis, IN; event MON810, Cry1Ab protein, Monsanto Company, St. Louis, MO; and event NK603, mEPSPS protein confers glyphosate herbicide tolerance, Monsanto Co.);
2) Cry1F × Cry1Ab pyramid (same as Treatment 1) plus a blended refuge non-Bt hybrid with only NK603;
3) Bt11 × MIR162 × GA21 pyramid (event Bt11, Cry1Ab protein; event MIR162, Vip3Aa20 protein; and event GA21 PAT protein confers glufosinate herbicide tolerance, all Syngenta, Research Triangle Park, NC);
4) Cry1F with events TC1507 × NK603;
5) and 7) two redundant treatments of non-Bt structured refuge containing herbicide tolerance only (event NK603); and
6) Cry1Ab containing events MON810 × NK603.

Pioneer® Brand hybrids with a relative maturity of 120 d from the 31D58 hybrid platform were used in all treatments (Treatment 1 = 31D56; Treatment 2 = 31D56 + refuge 31D57; Treatment 3 = unnumbered hybrid; Treatment 4 = 31D59; Treatments 5 and 7 = 31D57; Treatment 6 = 31D62). Treatments 5 and 7 were redundant non-Bt treatments.

Experimental details varied modestly among locations (Table 1). A randomized block design was used with four replications. Plots varied among locations from 5.3 to 12.2 m in length and were four rows wide. Distance between rows also varied among locations from 76 to 91 cm. Both planting density and seedling thinning were used to achieve a target population of ∼79,000 plants per ha across all experimental locations.

Seeds were supplied by DuPont Pioneer and contained 0.5 mg a.i./kernel thiamethoxam (Cruiser® 500, Syngenta Crop Protection, Greensboro, NC) for secondary pest management. Planting time was generally within the optimum range of dates recommended by

State Cooperative Extension guidelines in 2010, but up to 3 wk later than the optimal dates at some locations in 2011 because of weather conditions. Modifications to the general methodology and seed insecticide treatments were made in the lesser cornstalk borer experiment.

In 2010, refuge plants (non-Bt) in Treatment 2 (Cry1F × Cry1Ab plus non-Bt) were hand-planted immediately following planting of the Bt-hybrid by placing each seed approximately 5 cm to one side of the row and marked with a flag or small wooden stake so the non-Bt refuge plant could be readily identified after emergence. Non-Bt refuge seeds were separated by at least 1 m down the row and were planted at least 60 cm from the end of the rows. At plant emergence, any seedling plant within the row that emerged directly adjacent to the hand-planted refuge seed was removed to minimize plant crowding. In 2011, refuge seeds were pre-blended and incorporated with the Bt-producing hybrid at planting, so that refuge seeds were randomized within the plot. Refuge plants in 2011 were positively identified during the V5–V8 stage (Ritchie et al. 1982) by applying a mixture containing 1–2% v/v glufosinate (Liberty 280 SL, Bayer CropScience), 14–28% latex paint (brand and formulation variable), and water to the axial leaf surface of the outermost one-fourth portion of one of the two youngest fully emerged leaves on each plant in Treatment 2. Three to seven days after painting, plants were inspected and those exhibiting chlorotic tissue in the area of painting due to glufosinate injury were identified as non-Bt refuge plants and were marked with flagging tape.

Natural infestations of at least one of the target pests took place in each experiment, except for fall armyworm in KY and sugarcane borer in LA. At Lexington, KY, artificial infestations of fall armyworm from the University of Kentucky were applied on 26 June 2010 and 1 July 2011 at the rate of approximately 20 neonates per whorl in corn cob grits (Wiseman et al. 1980). At Alexandria, LA, artificial infestations of sugarcane borer were used for the two trials in 2010 and 2011. For the trial in 2010, five neonates (<24-h-old) per plant were manually infested in the collar of the leaf directly above the uppermost ear of the plant on 6 August 2010 at VT-R1 plant stages. For the trial in 2011, two insect infestations were applied. In the first infestation, six neonates were manually infested into the whorl on 3 July at 2011. In the second infestation, 10 neonates were placed in the collar of the leaf directly above the uppermost ear of the plant on 14 July 2011 at the VT-R1 planting stages.

The lesser cornstalk borer experiment in Burke Co., GA, was replicated six times during 2011. Seed in Treatments 1–6 was planted as seed without an insecticide seed treatment to measure the effect of non-Bt and Bt traits, while Treatment 7 "non-Bt check 2" (structured refuge) was seed that was treated with 0.25 mg a.i./kernel thiamethoxam (Cruiser® 5FS, Syngenta Crop Protection, Greensboro, NC). Plantings targeting lesser cornstalk borer occurred later than the optimum planting time to enhance infestation. Plot maintenance followed local Cooperative Extension guidelines, except that insect populations were encouraged by the use of cultural methods, such as later planting, high rates of irrigation, and fertilization.

Plant Injury Measurements. Leaf and ear ratings were collected on 20 consecutive plants within the treatment row with the first plant being selected at random, but not from the five plants from the end of the row. The exception was for Treatment 2 (Cry1F × Cry1Ab plus non-Bt blended refuge). For this treatment, in 2010, with some exceptions (Table 1), there were four refuge plants in each row and 16 Bt plants (two on either side of the refuge plant) with non-Bt plants and Cry1F × Cry1Ab plants sampled as five-plant clusters (Bt/Bt/non-Bt/Bt/Bt). In 2011, the number of refuge plants in each plot of the blended refuge treatment varied from three to five, for an approximate average of four, plus the concomitant Cry1F × Cry1Ab on either side sampled. When leaf feeding occurred in whorl-stage plants, ratings were performed when ~99% of the larvae had terminated feeding in the non-Bt structured refuge treatments. Leaf feeding injury was evaluated on a reverse 9–1 scale (*viz.*, Pioneer research protocol) and then converted to the Davis et al. (1992) 1–9 scale (1 = lowest injury, 9 = highest injury).

Ear injury measurements were taken when ~99% of the larvae had terminated feeding in the non-Bt structured refuge treatments. Injury was assessed by measuring the total square cm of corn earworm-attributed feeding to the primary ear kernels. A record of kernel consumption was limited to corn earworm alone. Although fall armyworm was present for some of the leaf ratings and will consume kernels, this species was not observed on ears either year of the experiment.

When stalk-tunneling species infested the plots, stalks from 10 plants in each plot were split at R5–R6. The lower eight internodes of each stalk (counting from the soil surface upward) were split longitudinally into two halves and total length of larval tunneling was measured in cm.

Plants were thinned to a uniform stand at the VC stage and evaluated for injury at 20–30 days after planting in the lesser cornstalk borer experiment. The number and frequency of dead and visibly injured plants was recorded on several dates in the early-vegetative stages (V3-V4) (Table 1). Plants were inspected and determined to be alive, dead, or injured, which included plants with "dead heart" (i.e., young whorl leaves dead, indicating a dead growing point, while older leaves may still be alive). At the final sampling date, all plants were dug and inspected for injury by lesser cornstalk borer. Injury was recorded if it penetrated the stem, but not if it injury was superficial.

Data Analyses. Treatment comparisons from each site across all years were used in separate analyses for the measurements of leaf injury from fall armyworm and corn earworm, kernel area consumed by corn earworm, length of stalk tunneled by southwestern corn borer or sugarcane borer, and percent dead or injured plants by lesser cornstalk borer. Data for all variables were averaged for each plot across plants or ears (sub-samples) prior to analysis. A generalized linear mixed-models approach (PROC GLIMMIX, SAS Institute 2008) was used because the data were not normally distributed. The fixed factor in the first set of analyses was treatment, which included all seven treatments [1): Cry1F × Cry1Ab; 2) Cry1F × Cry1Ab plus non-Bt blended refuge; 3) Cry1Ab × Vip3Aa20; 4) Cry1F; 5 & 7) redundant non-Bt structured refuge; and 6) Cry1Ab], with location and block modeled as random factors. Location was chosen as a random factor in the analysis so that the results were representative across the southern United States, rather than specific to a single location. A Poisson distribution was found to be the best fit for all models, but the data were over-dispersed (Littell et al. 2006). Therefore, a second random statement was added to include an over-dispersion parameter (RANDOM _RESIDUAL_). Analyses were performed with leaf injury, kernel area consumed, stalk tunneling by southwestern corn borer, stalk tunneling by sugarcane borer, and plants injured or killed by lesser cornstalk borer as the single dependent variable in each analysis. Data on the length of stalk tunneled by sugarcane borer ($y = \sqrt{(x + \frac{1}{100})}$) were transformed before analysis (Zar 1999).

In addition, separate analyses for each measurement were performed using the data taken from Treatment 2 (Cry1F × Cry1Ab plus non-Bt blended refuge), Treatment 1 (Cry1F × Cry1Ab), and Treatments 5 and 7 (non-Bt structured refuge). This analysis was termed "blended refuge versus structured refuge." A generalized linear mixed-models approach was used again (PROC GLIMMIX), with refuge type as the fixed factor. These included the Cry1F × Cry1Ab plants from Treatment 2, the non-Bt plants from Treatment 2, and plants from the two non-Bt structured refuge Treatments 5 and 7. The same random factors, including the over-dispersion parameter, were used as above and analyses were performed using the same dependent variables. For all analyses, mean separations were analyzed for statistical significance using Tukey's honestly significant difference test. Values were considered significantly different for all analyses when $\alpha < 0.05$.

Results

Plant-injury measurements for single-Bt hybrids (Cry1Ab and Cry1F), pyramided Bt hybrids (Cry1F × Cry1Ab and Cry1Ab × Vip3Aa20), and pyramided Cry1F × Cry1Ab plus non-Bt blended refuge were collected at 17 locations across 12 southern states during a two-year period (Table 1). At least one treatment was significantly different from others for every measurement (Table 2).

Leaf Injury. Leaf injury (1–9 scale) caused by either corn earworm or fall armyworm was minimal for the pyramided Bt treatments (Cry1F × Cry1Ab and Cry1Ab × Vip3Aa20) and pyramid with blended refuge (Cry1F × Cry1Ab plus non-Bt); leaf injury was not statistically different from the single Cry1F treatment and was higher in Treatment 6 with the single Cry1Ab treatment. Both of the non-Bt structured refuge treatments had nearly identical amounts of leaf injury, and the injury for each was significantly greater than any treatment containing a Bt protein.

Kernel Injury. Kernel injury—measured as cm^2 of kernels consumed by corn earworm larvae (Table 2)—for the pyramided (Cry1Ab × Vip3Aa20) treatment averaged 1.03 cm^2 injured kernels during the two years of testing across 17 locations. This level of injury was significantly less than that in other Bt treatments. The pyramided (Cry1F × Cry1Ab) treatment had nearly sixfold more kernel injury than the pyramided (Cry1Ab × Vip3Aa20) treatment, but the injury was not significantly different from the single-trait Cry1Ab treatment. Treatment 4, the single-trait Cry1F, had the highest corn earworm kernel injury of any Bt treatment, averaging 9.44 cm^2, and was not statistically different from the injury in either of the non-Bt structured refuge treatments.

Stalk Injury. Stalk injury from southwestern corn borer was measured in five and four states during 2010 and 2011, respectively (Table 1). All Bt treatments, both the single traits (Cry1Ab or Cry1F) and pyramided traits (Cry1F × Cry1Ab or Cry1Ab × Vip3Aa20), including the blended refuge, effectively minimized stalk injury from southwestern corn borer (Table 2). Mean stalk injury did not exceed 0.8 cm of tunneling and was not statistically different among any Bt treatment. In sharp contrast, stalk tunneling from southwestern corn borers averaged 37.1 and 50.6 cm in the two non-Bt structured refuge treatments.

Stalk injury from sugarcane borer was measured in Louisiana during both years. A similar trend was seen in stalk tunneling caused by sugarcane borer, although the intensity of the injury was much less than the southwestern corn borer injury. Injury to all Bt treatments, whether as single or pyramided traits or in a blended refuge, was minimal and not significantly different from one another. The larvae created significantly longer tunnels in the two non-Bt structured refuge treatments, which averaged 4.79 and 8.39 cm (Table 2).

Dead or Injured Plants from Lesser Cornstalk Borer. Seedling plant mortality from lesser cornstalk borer was measured in Burke, GA, during both years (Table 1). No statistical difference in plant injury or mortality was detected among either the single- or pyramided-Bt treatments; average injury or mortality was low and ranged from 1.93 to 7.62% (Table 2). Plant injury or plant mortality in the non-Bt structured refuge treatment without an insecticidal seed treatment averaged 41.33% and was significantly greater than all other treatments, including the second non-Bt hybrid structured refuge treatment, which was treated with thiamethoxam. The injury or mortality in the insecticide-treated non-Bt structured refuge treatment was not different from the plant injury or mortality observed in two of the Bt treatments: Cry1F, and pyramided Cry1Ab × Vip3Aa20.

Blended Refuge versus Structured Refuge. Non-Bt plants within Treatment 2 (pyramided Cry1F × Cry1Ab plus non-Bt blended refuge) had

Table 2. Plant injury (means ± SE) from corn earworm, fall armyworm, southwestern corn borer, sugarcane borer, and lesser cornstalk borer to corn expressing single- or pyramid-Bt traits and non-Bt corn across 12 southern states, 2010–2011[a]

Cry protein and refuge scenario	Leaf[b]	Kernel[c]	Stalk[d]	Stalk[e]	Percent dead or injured[f]
Cry1F × Cry1Ab pure stand	1.43 ± 0.09a	6.01 ± 0.55b	0.15 ± 0.05a	0.05 ± 0.04a	1.93 ± 0.64a
Cry1F × Cry1Ab blended refuge[g]	1.70 ± 0.11a	6.28 ± 0.53b	1.80 ± 0.51a	0.68 ± 0.30b	18.78 ± 3.38bc
Cry1Ab × Vip3Aa20 pure stand	1.21 ± 0.05a	1.03 ± 0.20a	0.80 ± 0.39a	0.003 ± 0.003a	7.62 ± 3.78ab
Cry1F pure stand	1.38 ± 0.08ab	9.44 ± 0.81cd	0.45 ± 0.20a	0.01 ± 0.01a	4.83 ± 1.40ab
Cry1Ab pure stand	1.78 ± 0.15b	7.69 ± 0.68bc	0.16 ± 0.05a	0.00 ± 0.00a	2.77 ± 1.44a
Non-Bt check 1 structured refuge	3.00 ± 0.22c	11.3 ± 1.05d	50.60 ± 20.54b	4.79 ± 0.93c	41.33 ± 5.40c
Non-Bt check 2 structured refuge	3.01 ± 0.23c	10.78 ± 0.89d	37.12 ± 13.69c	8.39 ± 1.93c	14.66 ± 3.07b
	($F = 28.99$; df = 6, 373.4; $P < 0.0001$)	($F = 13.60$; df = 6, 820.2; $P < 0.0001$)	($F = 46.64$; df = 6, 266.2; $P < 0.0001$)	($F = 47.51$; df = 6, 76; $P < 0.0001$)	($F = 15.66$; df = 6, 123; $P < 0.0001$)

[a] Different letters within a column represent means that are significantly different ($P < 0.05$) by Tukey's HSD procedure.

[b] Leaf rating on a 1–9 scale (Davis et al. 1992) from injury caused by fall armyworm and/or corn earworm. Depending on the year and location, either fall armyworm, corn earworm, or a combination of both pests caused the injury. This scale ranged from 1 = no visible injury or small pin holes visible on a few leaves to 9 = most of leaves with long lesions.

[c] Kernels (cm^2) consumed by corn earworm larvae.

[d] Linear cm of stalk tunneled by southwestern corn borer larvae.

[e] Linear cm of stalk tunneled by sugarcane borer larvae.

[f] Plants dead or injured (percent) in the whorl from lesser cornstalk borer injury, where all seeds were planted *without* an insecticidal seed treatment, with the exception non-Bt check 2 structured refuge treatment (Treatment 7, treated with 0.25 mg a.i./kernel thiamethoxam).

[g] Consists of a five-plant cluster (non-Bt plant with two Bt plants on either side).

Table 3. Plant injury (means ± SE) from five lepidopteran pests for all locations and years to Cry1F × Cry1Ab corn in a structured refuge and with blended non-Bt refuge plants, 2010-2011[a]

Cry protein and refuge scenario	Leaf[b]	Kernel[c]	Stalk[d]	Stalk[e]	Percent dead or injured[f]
Cry1F × Cry1Ab plants within refuge plant blend[g]	1.64 ± 0.18a	5.29 ± 0.51b	0.19 ± 0.08a	0.01 ± 0.01a	13.92 ± 3.80c
Non-Bt plants within Cry1F × Cry1Ab blend[h]	2.83 ± 0.25b	10.96 ± 0.94a	7.84 ± 2.74b	5.47 ± 1.38a	82.50 ± 10.31a
Non-Bt check 1 structured refuge	3.00 ± 0.23b	11.30 ± 1.05a	50.60 ± 20.54b	4.79 ± 0.93a	41.33 ± 5.40b
Non-Bt check 2 structured refuge	3.01 ± 0.23b	10.78 ± 0.89a	37.12 ± 13.69b	8.39 ± 1.93a	14.66 ± 3.07c
	($F = 12.79$; df = 3, 388; $P < 0.0001$)	($F = 41.95$; df = 3, 425.9; < 0.0001)	($F = 11.75$; df = 3, 110.4; $P < 0.0001$)	($F = 2.46$; df = 3, 41.89; $P = 0.0762$)	($F = 17.51$; df = 3, 56.08; $P < 0.0001$)

[a] Different letters within a column represent means that are significantly different ($P < 0.05$) by Tukey's HSD procedure.

[b] Leaf rating on a 1–9 scale (Davis et al. 1992) from injury caused by fall armyworm and/or corn earworm. Depending on the year and location, either fall armyworm, corn earworm, or a combination of both pests caused the injury. This scale ranged from 1 = no visible injury or small pin holes visible on a few leaves to 9 = most of leaves with long lesions.

[c] Kernels (cm^2) consumed by corn earworm larvae.

[d] Linear cm of stalk tunneled by southwestern corn borer larvae.

[e] Linear cm of stalk tunneled by sugarcane borer larvae.

[f] Plants dead or injured (percent) in the whorl from lesser cornstalk borer injury. All seed were planted with an insecticidal seed treatment, with the exception of non-Bt check 1 structured refuge (Treatment 5) for lesser cornstalk borer.

[g] Mean of two Bt plants adjacent to a Bt plant.

[h] Mean of non-Bt plants flanked by Bt plants.

similar injury from most pests when compared to the non-Bt structured refuge treatments (Table 3). The one exception was lesser cornstalk borer, where 82.5% of non-Bt plants were injured or killed within the pyramided Cry1F × Cry1Ab (Treatment 2), compared to 41.33% (without a seed treatment), and 14.66% (with seed treatment) of plants in the non-Bt structured refuge treatments. Nearly 14% of pyramided Cry1F × Cry1Ab plants in Treatment 2 were injured or killed by lesser cornstalk borer, which was similar to the frequency of dead or injured plants in one of the non-Bt structured refuge treatments.

For nearly all insect pests and injury types measured, Cry1F × Cry1Ab (Treatment 2) plants had significantly less injury than the companion blended non-Bt plants. The single exception was the length of stalk tunneled by sugarcane borer; there was no significant difference between blended Cry1F × Cry1Ab plants and non-Bt plants (Table 3).

Discussion

Lepidopteran pests in southern U.S. corn production did not differentially injure non-Bt plants blended with Cry1F × Cry1Ab plants compared to non-Bt plants in a structured refuge with a seed treatment. One exception was the lesser cornstalk borer, which injured or killed 42% more plants in a blended seed mixture, compared to a structured refuge of non-Bt plants. Although neighboring non-Bt plants within the pyramided Cry1F × Cry1Ab plus non-Bt blended refuge treatment were more injured by pests than the plants producing two Bt proteins, a pure stand of pyramided Cry1F × Cry1Ab effectively managed overall plant injury caused by fall armyworm and corn earworm to the leaf, kernel injury caused by corn earworm on the ear, and injury caused by tunneling of southwestern corn borer. The pyramided Cry1Ab × Vip3Aa20 structured refuge (Treatment 3) was the most effective treatment tested against all insect pests. Pyramided dual-gene Bt treatments were more effective in protecting the plants from fall armyworm and corn earworm compared to the single-trait Bt treatments.

Results for both single Bt traits were consistent with findings from previous researchers. For example, the production of Cry1F did not reduce corn earworm injury (Buntin 2008), unless it was pyramided with another trait (Siebert et al. 2012), in this case, Cry1Ab. Leaf feeding due to fall armyworm or corn earworm in combination was documented in some, but not all trials. Leaf feeding data were analyzed as a single factor, regardless of whether it was from fall armyworm and/or corn earworm. In contrast, the vast majority of kernel injury was from corn earworm, without the confounding presence of another pest. Therefore, leaf ratings were the best indicators of fall armyworm injury. In these trials, hybrids containing Cry1F were effective to manage fall armyworm, similar to previous study in the same geography (Buntin 2008), despite the fact that resistance to this protein was present in populations of fall armyworm in Puerto Rico (Storer et al. 2010a). All hybrids containing a single Bt trait or pyramided Bt traits were effective against the southwestern corn borer, sugarcane borer, and lesser cornstalk borer in a pure stand, which agrees with previous observations (Buschman et al. 2001, Vilella et al. 2002, Royer et al. 2003, Castro et al. 2004, Siebert et al. 2012). The pyramided Cry1Ab × Vip3Aa20 structured refuge treatment was the most effective or among the most effective treatment tested for all insect pests. This Bt pyramid is highly effective against fall armyworm, corn earworm, southwestern corn borer, sugarcane borer (Dively 2005, Yang et al. 2013), and lesser cornstalk borer, as demonstrated in the current study.

Blended refuge was not as effective when compared to a pure stand of Cry1F × Cry1Ab when sugarcane borer and lesser cornstalk borer were present. Both pests are relatively minor in incidence across the southern United States. Hybrids containing Cry1F have been shown to effectively reduce injury from these pests (Seibert et al. 2012). Therefore, we would expect less injury in any hybrid with this protein relative to a non-Bt hybrid. However, the presence of non-Bt plants within the blended refuge likely contributed to the greater injury observed in a blended as opposed to a pure stand of Cry1F × Cry1Ab. For lesser cornstalk borer, 82.5% of the non-Bt plants and 13.92% of plants producing two Bt proteins (Cry1F × Cry1Ab) were killed in the pyramided Cry1F × Cry1Ab plus non-Bt blended refuge treatment. This was significantly higher than the percentage of plants injured or killed in the Cry1F × Cry1Ab. Non-Bt plants within the blended refuge were much more injured than plants expressing Bt toxins. One possibility is that separate refuges are more effective in managing pests with relatively mobile larvae, such as lesser cornstalk borer, than are blended refuges. The stalk-boring larvae of European corn borer can move among adjacent corn plants and do so with more frequency when exposed to corn plants producing Cry1Ab (Prasifka et al. 2009, Razze and Mason 2012) and those producing both Cry1F × Cry1Ab (Razze and Mason 2012). Larvae of sugarcane borer also showed the ability to move from infested plants to at least four plants away and to adjacent rows, but the majority remained within the infested row (Wangila et al. 2013). Published studies are available for intraplant movement for some of the stalk-boring pests in this study [*viz.*, for southwestern corn borer (Davis et al. 1972, Chippendale 1978)], but not for interplant movement. Similarly, published data are available for the leaf and ear-feeding pests in this study for intraplant movement [*viz.*, for corn earworm (Wiseman et al. 1978, Zalucki et al. 2002) and fall armyworm (Yang et al. 1993)], but not interplant movement. Lesser cornstalk borer feeds differently than the other pests in this study, because it feeds just below the surface of the soil in silken tubes that often radiate out from the feeding site (Tippins 1982). Larvae can move so readily that it is commonly called the jumping caterpillar, given its habit of contorting and jumping when disturbed. Given the feeding habit and relative mobility, it is possible that the blended refuge was not effective for managing lesser cornstalk borer because larvae could readily move between Bt-producing and non-Bt plants. An insecticidal seed treatment at a low-labeled rate effectively reduced the number of dead or injured plants to levels similar to those observed in the Bt treatments, with the exceptions of the Cry1F × Cry1Ab and Cry1Ab. More information is needed on the interplant movement of the larval pests of corn, especially between Bt-producing and adjacent non-Bt plants.

When Cry1Ab and Cry1F were first commercially introduced, neither Bt protein was intended to manage corn earworm or fall armyworm in corn. Rather, these proteins were incorporated into the plant for the purpose of reducing European corn borer and southwestern corn borer population densities (Storer et al. 2001). However, these proteins do reduce larval growth of corn earworm and can cause some mortality (Sims et al. 1996, Williams et al. 1998, Storer et al. 2001). The deployment of single Bt traits in both corn and cotton that do not kill a high proportion of the target insects would possibly increase resistance. Pyramiding both the traits that produce Cry1F and Cry1Ab appeared to increase mortality in the studies presented here, as measured indirectly by a decrease in plant injury. Hence, this tactic could serve to delay resistance. According to this logic and the results of this study, Cry1Ab × Vip3Aa20 would be expected to have the greatest durability against corn earworm among the trait combinations tested. These results also corroborate the findings of Burkness et al. (2010) for Cry1Ab × Vip3A pyramid against corn earworm. Finally, if the blended refuge tactic is to extend durability of the Bt traits, larvae must receive a sufficiently high dose of Bt to reduce the chance that they can complete development by moving between Bt and non-Bt plants. Furthermore, the dose of the toxin must be high enough after cross-pollination between Bt and non-Bt plants to kill insects that feed on kernels. The potential of this occurrence remains unknown, as the proportion of individuals with a resistant allele is also unknown.

In conclusion, for selected southern U.S. insect corn pests evaluated, hybrids with pyramided Bt traits were more effective for managing fall armyworm and corn earworm. Both single-Bt trait and pyramided-Bt trait hybrids were effective against southwestern corn borer, sugarcane borer, and lesser corn stalk borer. Cry1Ab × Vip3Aa was the most effective pyramid or among the most effective for all insect pests. The efficacy of plants containing Cry1F × Cry1Ab was not influenced by a blended refuge scenario, compared to plots mimicking a pure stand for major southern U.S. pests. Non-Bt plants within the blended refuge did not differ significantly in injury than non-Bt plants (except with lesser cornstalk borer) in plots mimicking a structured refuge. These data support the concept that a blended non-Bt refuge would not lessen resistance management based on the refuge approach for the southern corn insect complex considered in this study. Without considering the role of grower compliance in planting separate refuge, these data suggest that it is likely that the period for development of resistance to these traits would remain static, compared to the traditional structured refuge in the southern United States.

Acknowledgments

This study was funded in part by DuPont Pioneer. We thank key assistants in the field project: Darwin Anderson (Texas A&M AgriLife Research), Malcomb Pegues (Alabama Agricultural Experiment Station), S. Roberson and T. Clifton Moore (North Carolina State University), Audra Miller and Gene Windham (University of Missouri), William Griggs (Clemson University), Kevin Ford, Tanya Briggs, and Neal Wright (Delta Research and Extension Center, Mississippi State University), N.D. Kemp and W. R. Slaughter, Jr. (University of Georgia), and Karla Emfinger, Thomas Williams, and Jessica Moore (Louisiana State University AgCenter). The suggestions of Susan Moser, Herb Eichenseer, and Tim Nowatzki (DuPont Pioneer), and three anonymous reviewers are greatly appreciated; their constructive comments and edits improved the manuscript.

References Cited

ABSTC (Agricultural Biotechnology Stewardship Technical Committee). 2013. 2012 ABSTC insect resistance management compliance assurance program (CAP) report. (http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2011-0922-0040) (accessed 4 June, 2013).

Bates, S. L., J. -Z. Zhao, R. T. Roush, and A. M. Shelton. 2005. Insect resistance management in GM crops: past, present, and future. Nat. Biotechnol. 23: 57–62.

Buntin, G. D., K. L. Flanders, and R. E. Lynch. 2004. Assessment of experimental Bt events against fall armyworm and corn earworm in field corn. J. Econ. Entomol. 97: 259–264.

Buntin, G. D. 2008. Corn expressing Cry1Ab or Cry1F endotoxin for fall armyworm and corn earworm (*Lepidoptera: Noctuidae*) management in field corn for grain production. Fla. Entomol. 91: 523–530.

Burkness, E. C., G. Dively, T. Patton, A. C. Morey, and W. D. Hutchison. 2010. Novel Vip3A *Bacillus thuringiensis* (*Bt*) maize approaches high-dose efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae) under field conditions: implications for resistance management. GM Crops 1: 337–343.

Burkness, E. C., P. K. O'Rourke, and W. D. Hutchison. 2011. Cross-pollination of nontransgenic corn ears with transgenic Bt corn: efficacy against Lepidopteran pests and implications for resistance management. J. Econ. Entomol. 104: 1476–1479.

Buschman, L., P. Sloderbeck, and M. Witt. 2001. Efficacy of Cry1F corn for the control of southwestern corn borer and corn earworm, 2000. Arthrop. Manag. Tests 26: M2.

Castro, B. A., B. R. Leonard, and T. J. Riley. 2004. Management of feeding damage and survival of southwestern corn borer and sugarcane borer (Lepidoptera: Crambidae) with *Bacillus thuringiensis* transgenic field corn. J. Econ. Entomol. 97: 2106–2116.

Chilcutt, C. F., and B. E. Tabashnik. 2004. Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize. Proc. Natl. Acad. Sci. 101: 7526–7529.

Chippendale, G. M. 1978. Behavior associated with the larval diapause of the southwestern corn borer, *Diatraea grandiosella*: Probable involvement of juvenile hormone. Ann. Entomol. Soc. Am. 71: 901–905.

Davis, F. M., C. A. Henderson, and G. E. Scott. 1972. Movements and feeding of larvae of the southwestern corn borer on two stages of corn growth. J. Econ. Entomol. 65: 519–521.

Davis, F. M., S. S. Ng, and W. P. Williams. 1992. Visual rating scales for screening whorl-stage corn for resistance to fall armyworm. Miss. Agric. For. Exp. Stn. Tech. Bull. 186: 1–9.

Dively, G. P. 2005. Impact of transgenic VIP3Aa20 × Cry1Ab lepidopteran-resistant field corn on the nontarget arthropod community. Environ. Entomol. 34: 1267–1291.

Flanders, K. L. 2013. Insect pest management. pp. 1–30. *In* IPM corn insect, disease, nematode and weed control recommendations for 2013, IPM-0428. Alabama Coop. Ext. Sys., Alabama, A&M Univ. and Auburn Univ., Auburn, AL.

Gould, F. 1986. Simulation models for predicting durability of insect-resistant germ plasm: a deterministic diploid, two-locus model. Environ. Entomol. 15: 1–10.

Hensley, S. D. 1971. Management of sugarcane borer populations in Louisiana, a decade of change. Entomophaga 16: 133–146.

Hutchison, W. D., E. C. Burkness, P. D. Mitchell, R. D. Moon, T. W. Leslie, S. J. Fleischer, M. Abrahamson, K. L. Hamilton, K. L. Steffey, M. E. Gray. et al. 2010. Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330: 222–225.

Jaffe, G. 2009. Complacency on the farm. Center for Insect Science in the Public Interest, Washington, DC. (http://cspinet.org/new/pdf/complacencyonthefarm.pdf).

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for mixed models, 2nd ed. SAS Institute, Cary, NC.

(NASS) National Agricultural Statistics Service. 2013. Acreage. ISSN: 1949-1522. U. S. Dept. Agric., Nat'l. Agric. Stat. Serv., Washington, DC.

Onstad, D. W., P. D. Mitchell, T. M. Hurley, J. G. Lundgren, R. P. Porter, C. H. Krupke, J. L. Spencer, C. D. DiFonzo, T. S. Baute, R. L. Hellmich. et al. 2011. Seeds of change: corn seed mixtures for resistance management and IPM. J. Econ. Entomol. 104: 343–352.

Prasifka, J. R., R. L. Hellmich, A.L.B. Crespo, B. D. Siegfried, and D. W. Onstad. 2009. Video-tracking and on-plant tests show Cry1Ab resistance influences behavior and survival of neonate *Ostrinia nubilalis* following exposure to Bt maize. J. Insect Behav. 23: 1–11.

Razze, J. M., and C. E. Mason. 2012. Dispersal behavior of neonate European corn borer (Lepidoptera: Crambidae) on Bt corn. J. Econ. Entomol. 105: 1214–1223.

Ritchie, S. W., J. J. Hanway, and G. O. Benson. 1982. How a corn plant develops. Iowa St. Univ. Coop. Ext., Spec. Rep. 48.

Royer, T. A., K. L. Giles, D. Kastl, R. Kochenower, and V. B. Langston. 2003. Evaluation of transgenic Bt-corn hybrids for control of southwestern corn borer, 2002. Arthro. Manag. Tests 26: M2.

SAS Institute. 2008. SAS/STAT 9.2 User's Guide, 2nd ed., SAS Institute, Cary, NC.

Siebert, M. W., S. P. Nolting, W. Hendrix, S. Dhavala, C. Craig, B. R. Leonard, S. D. Stewart, J. All, F. R. Musser, G. D. Buntin, and L. Samuel. 2012. Evaluation of corn hybrids expressing Cry1F, Cry1A.105, Cry2Ab2, Cry34Ab1/Cry35Ab1, and Cry3Bb1 against southern United States insect pests. J. Econ. Entomol. 105: 1825–1834.

Sims, S. R., J. C. Pershing, and B. J. Reich. 1996. Field evaluation of transgenic corn containing a *Bacillus thuringiensis* Berliner insecticidal protein gene against *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Entomol. Sci. 31: 340–346.

Storer, N. P., J. W. van Duyn, and G. G. Kennedy. 2001. Life history traits of *Helicoverpa zea* (Lepidoptera: Noctuidae) on non-Bt and Bt transgenic corn hybrids in eastern North Carolina. J. Econ. Entomol. 94: 1268–1279.

Storer, N. P., J. M. Babcock, M. Schlenz, T. Meade, G. D. Thompson, J. W. Bing, and R. M. Huckaba. 2010a. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. J. Econ. Entomol. 103: 1031–1038.

Storer, N. P., G. D. Thompson, and G. P. Head. 2010b. Application of pyramided traits against Lepidoptera in insect resistance management for Bt crops. GM Crops Food 3: 154–162.

Tippins, H. H. 1982. A review of information on the lesser cornstalk borer *Elasmopalpus lignosellus* (Zeller). Ga. Agric. Exp. Stn. Spec. Publ. 17.

Vilella, F.M.F., J. M. Waquil, E. F. Vilella, P. A. Viana, R. E. Lynch, and J. E. Foster. 2002. Resistance of Bt transgenic maize to lesser cornstalk borer (Lepidoptera: Pyralidae). Fla. Entomol. 85: 652–653.

Wangila, D. S., B. R. Leonard, M. N. Ghimire, Y. Bai, L. Zhang, Y. Yang, K. D. Emfinger, G. P. Head, F. Yang, Y. Niu. et al. 2013. Occurrence and larval movement of *Diatraea saccharalis* (Lepidoptera: Crambidae) in seed mixes of non-Bt and Bt pyramid corn. Pest Manag. Sci. 69: 1163–1172.

Williams, W. P., P. M. Buckley, J. B. Sagers, and J. A. Hanten. 1998. Evaluation of transgenic corn for resistance to corn earworm (Lepidoptera: Noctuidae), fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer (Lepidoptera: Crambidae) in a laboratory bioassay. J. Agric. Entomol. 14: 105–112.

Wiseman, B. R., N. W. Widstrom, and W. W. McMillian. 1978. Movement of corn earworm larvae on ears of resistant and susceptible corns. Environ. Entomol. 7: 777–779.

Wiseman, B. R., F. M. Davis, and J. E. Campbell. 1980. Mechanical infestation device used in fall armyworm plant resistance programs. Fla. Entomol. 63: 425–432.

Yang, G. B. R. Wiseman, and K. E. Espelie. 1993. Movement of neonate fall armyworm (Lepidoptera: Noctuidae) larvae on resistant and susceptible genotypes of corn. Environ. Entomol. 22: 547-553

Yang, F., F. Huang, J. A. Qureshi, B. R. Leonard, Y. Niu, L. Zhang, and D. S. Wanglia. 2013. Susceptibility of Louisiana and Florida populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to transgenic Agrisure® Viptera™ 3111 corn. Crop Prot. 50: 37–39.

Zalucki, M. P., A. R. Clarke, and S. B. Malcolm. 2002. Ecology and behavior of first instar larval Lepidoptera. Annu. Rev. Entomol. 47: 361–393.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ.

Received 16 May 2014; accepted 2 October 2014.