

Impact of Corn Earworm (Lepidoptera: Noctuidae) on Field Corn (Poales: Poaceae) Yield and Grain Quality

Jenny L. Bibb,^{1,7} Donald Cook,² Angus Catchot,¹ Fred Musser,¹ Scott D. Stewart,³ Billy Rogers Leonard,⁴ G. David Buntin,⁵ David Kerns,⁶ Tom W. Allen,² and Jeffrey Gore²

¹Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, P.O. Box 9775, Mississippi State, MS 39762, ²Delta Research and Extension Center, Mississippi State University, P.O. Box 197, Stoneville, MS 38776, ³The University of Tennessee, WTREC, 605 Airways Boulevard, Jackson, TN 38301, ⁴LAES Administration, Louisiana State University, 104 J Norman Efferson Hall, 110 LSU Union Square, Baton Rouge, LA 70803, ⁵Department of Entomology, University of Georgia, 1109 Experiment Street, Griffin, GA 30223, ⁶Department of Entomology, Texas A&M University, TAMU 2475, College Station, TX 77843, and ⁷Corresponding author, e-mail: j.bibb@msstate.edu

Subject Editor: John Trumble

Received 30 January 2018; Editorial decision 1 March 2018

Abstract

Corn earworm, *Helicoverpa zea* (Boddie), commonly infests field corn, *Zea mays* (L.). The combination of corn plant biology, corn earworm behavior in corn ecosystems, and field corn value renders corn earworm management with foliar insecticides noneconomical. Corn technologies containing *Bacillus thuringiensis* (Bt) Berliner (Bacillales: Bacillaceae) were introduced that exhibit substantial efficacy against corn earworm and may reduce mycotoxin contamination in grain. The first generation Bt traits in field corn demonstrated limited activity on corn earworm feeding on grain. The pyramided corn technologies have greater cumulative protein concentrations and higher expression throughout the plant, so these corn traits should provide effective management of this pest. Additionally, reduced kernel injury may affect physical grain quality. Experiments were conducted during 2011–2012 to investigate corn earworm impact on field corn yield and grain quality. Treatments included field corn hybrids expressing the Herculex, YieldGard, and Genuity VT Triple Pro technologies. Supplemental insecticide treatments were applied every 1–2 d from silk emergence until silk senescence to create a range of injured kernels for each technology. No significant relationship between the number of corn earworm damaged kernels and yield was observed for any technology/hybrid. In these studies, corn earworm larvae did not cause enough damage to impact yield. Additionally, no consistent relationship between corn earworm damage and aflatoxin contamination was observed. Based on these data, the economic value of pyramided Bt corn traits to corn producers, in the southern United States, appears to be from management of other lepidopteran insect pests including European and southwestern corn borer.

Key words: *Helicoverpa zea*, pyramided Bt's, maize, IPM, *Bacillus thuringiensis*

Corn earworm, *Helicoverpa zea* (Boddie), infests both field corn and sweet corn. The tolerances for larvae in fresh market sweet corn are very low (DePew 1966, Shelton et al. 2013). With the high value of fresh market sweet corn, corn earworm management with foliar insecticides is economically viable. Foliar insecticide applications must be targeted at eggs and newly emerged larvae before they enter the ear. Residual activity of foliar applications can be compromised by the rapid growth rate of corn silks. This can result in the presence of newly emerged unprotected silk within 1 d of an insecticide application. This combination of factors creates the need for multiple insecticide applications (12 to 40 treatments) to manage corn earworm and fall armyworm, *Spodoptera frugiperda* (J. E. Smith), in

commercially grown sweet corn (Janes and Greene 1969, Foster 1989). With the current value of field corn, this strategy is not economical, and corn earworm is not been considered an economic pest (USDA NASS 2012).

The first generation transgenic *Bacillus thuringiensis* (Bt) corn hybrids active against lepidopteran pests expressed a single Bt protein and were developed primarily to manage the corn borer complex, which includes sugarcane borer, *Diatraea saccharalis* (F.), European corn borer, *Ostrinia nubilalis* (Hübner), and southwestern corn borer, *Diatraea grandiosella* (Dyar) (Ostlie et al. 1997; Huang et al. 2006a,b). YieldGard Corn Borer and Herculex I are two of the most widely planted single-gene technologies, containing the

proteins Cry1Ab (YieldGard) and Cry1F (Herculex). The Herculex trait (Cry1F) also exhibits substantial activity against fall armyworm (Luo et al. 1999, Siebert et al. 2008a,b; Hardke 2011). The newest Bt corn trait combinations with lepidopteran pest activity now express multiple toxins, which are referred to as pyramided traits (Tabashnik et al. 2013). The pyramided Bt corn technologies with lepidopteran activity were introduced as an insecticide resistance management strategy (IRM) to maintain the durability of the technology and to improve efficacy against a broader range of lepidopteran pests (Gryspeirt and Grégoire 2012, Huang et al. 2014).

The value of enhanced corn earworm activity demonstrated by these new technologies is unclear because the impact of corn earworm infestations on field corn yield has not been fully investigated. The ability of the newer transgenic Bt corn technologies to reduce corn earworm injury has been demonstrated; however, yield responses directly associated with reductions in insect damage have been inconsistent in field corn (Buntin et al. 2001, 2004a; Buntin 2008; Reay-Jones and Wiatrak 2011; Bowen et al. 2014; Reay-Jones and Reising 2014). In addition to corn earworm control, these new Bt technologies may potentially reduce mycotoxin contamination in mature grain (Odvody et al. 2000, Buntin et al. 2001, Shelton et al. 2002, Williams et al. 2002).

Mycotoxins are the toxic secondary metabolites of certain fungi under specific conditions (Koenning and Payne 1999, Bennett and Klich 2003). They are an important regulatory concern worldwide due to their toxic and carcinogenic effects in both animals and humans (Wu 2006). Mycotoxin contamination of crops may cause economic losses of millions of dollars even during favorable seasons (Charmley et al. 1995). One of the most notable mycotoxins, with regard to corn production, is aflatoxin produced by the fungus *Aspergillus flavus* (Link) and several other species of *Aspergillus* (Hesseltine et al. 1966, Dorner et al. 1999, Hedayati et al. 2007). Aflatoxins are some of the most potent chemical liver carcinogens, and poisoning by aflatoxins (aflatoxicosis) is characterized by acute liver damage, edema, and possibly death resulting from extremely high doses (Wu 2006). Plant stresses caused by hot, dry weather, poor irrigation management, nutrient deficiency, and insect injury can be conducive to aflatoxin production (Jones et al. 1981, Dorner et al. 1999, Koenning and Payne 1999). By reducing insect injury (i.e., corn earworm), the new Bt technologies have the potential to reduce mycotoxin concentrations.

These new technologies could be extremely beneficial to corn growers in the southern United States. Therefore, there is a need to determine the impact and value of these technologies to Mid-southern field corn.

Materials and Methods

Impact of Corn Earworm on Field Corn Yield

Experiments were conducted at five locations, which included the Mississippi State University R. R. Foil Plant Science Farm, Starkville, MS; Delta Research and Extension Center, Stoneville, MS; University of Tennessee West Tennessee Experiment Station, Jackson, TN; LSU AgCenter Macon Ridge Research Station, Winnsboro, LA; and University of Georgia Bledsoe Research Station, Griffin, GA. Corn seed was planted both during and after the recommended planting period in 2011 and 2012 at each location to increase the probability of encountering persistent corn earworm infestations (McClure 2010, Larson 2012, Lee 2013, Anonymous 2016). Planting dates were each considered a separate trial. Planting and harvesting dates are detailed in Table 1. The treatments consisted of three transgenic

Table 1. Planting and harvest dates: during and after recommended planting date

Year	Location	Planting date	Harvest date
2011	Starkville, MS	April 12	Aug. 30
2011	Starkville, MS	June 1	Oct. 14
2011	Starkville, MS	June 21	Nov. 2
2012	Starkville, MS	Mar. 29	Aug. 30
2012	Starkville, MS	May 22	Oct. 9
2011	Stoneville, MS	April 8	Aug. 23
2011	Stoneville, MS	May 10	Sept. 9
2012	Stoneville, MS	April 5	Aug. 28
2012	Stoneville, MS	June 7	Sept. 24
2011	Jackson, TN	May 9	Sept. 6
2011	Jackson, TN	May 31	Oct. 4
2012	Jackson, TN	April 25	Sept. 11
2012	Jackson, TN	May 10	Sept. 23
2011	Winnsboro, LA	April 11	Aug. 12
2011	Winnsboro, LA	May 23	Sept. 21
2012	Winnsboro, LA	April 10	Aug. 24
2012	Winnsboro, LA	May 2	Sept. 10
2011	Griffin, GA	April 15	Aug. 25
2011	Griffin, GA	May 31	Sept. 27
2012	Griffin, GA	April 4	Sept. 12
2012	Griffin, GA	June 4	Oct. 10

Bt corn technologies, each expressing proteins active against lepidopteran pests including Herculex 1 (Cry1F, Dow AgroSciences, Indianapolis, IN; "P1615HR," Pioneer Hi-Bred, Johnston, IA), YieldGard (Cry1Ab, "DKC 69-40," Monsanto Company, St. Louis, MO), and Genuity VT Triple Pro (Cry1A.105 + Cry2Ab2, "DKC 67-88," Monsanto Company). Genuity VT Triple Pro also expresses the Cry3Bb1 protein, which is active against corn rootworm, *Diabrotica* spp., which were not economic pests at any test location. Hybrids (technologies) were arranged in a randomized complete block design with four replications. Plot size was eight rows, and row spacing ranged from 76.2 to 101.6 cm (30 to 40 inches) with a plot length of 9.1 to 15.2 m (30 to 50 ft). Individual rows of each plot were designated for specific data collection or plot isolation: rows one and eight were a border between treatments, rows two and three were used for in-season destructive sampling to record larval infestations (these rows were not treated with foliar insecticide and not harvested), rows four and five were not treated and harvested for grain yields, and rows six and seven were treated with foliar insecticides and harvested for grain yields.

In 2011, two rows (six and seven) in each plot of a trial were treated with either 0.105 kg ai/ha (0.094 lb ai/acre) of flubendiamide (Belt 45C, Bayer CropScience, Research Triangle Park, NC) or 0.075 kg ai/ha (0.067 lb ai/acre) of chlorantraniliprole (Prevathon 0.43SC, DuPont, Wilmington, DE) beginning at silk emergence (R1) and continuing until silk senescence (R4) (ca. 21 d) based on preference of the investigator. Foliar treatments applied by CO₂ backpack sprayer with the "spraying to wet" (40 GPA) method targeted the ears only and were made every 1–2 d. In 2012, foliar insecticide applications were made to the center two rows (six and seven) and consisted of flubendiamide at 0.105 kg ai/ha (0.094 lb ai/acre) every 1–2 d beginning at silk emergence (R1) until 5 d after silk emergence. On the fifth day of silking, 0.5 ml of a 0.2% solution of formulated flubendiamide plus 0.05% solution of methylated seed oil was injected into the area inside of the husk surrounding the silks and tip of each ear to further insect control in the ear. Injections were made using a cattle injection syringe (Allflex 25MR2 Repeater Syringe, 25 ml, Allflex USA, Inc., DFW Airport, TX) with the end

of a blunt needle (16 gauge). These treated rows along with corresponding nontreated rows in each plot provided a range of corn earworm injured ears within each plot. Twenty years from the destructive sampling rows were examined to determine natural lepidopteran species, numbers, and instar present at the R3 growth stage (milk). A second sample was collected at R4 growth stage (dough). Within 1–2 d before harvest, 50 ears each from the treated rows and the nontreated rows of each plot were examined for injured kernels. On the day of harvest, ears were examined on the stalks by peeling back the husks, then the treated and nontreated rows of each plot were mechanically harvested and grain yields were corrected to 15% moisture content. Fertilizer application, irrigation, and other agronomic practices, except insecticide sprays, were applied as per the recommendations of each state's Cooperative Extension Services (McClure 2010, Larson 2012, Lee 2013, Anonymous 2016).

Impact of Corn Earworm on Grain Quality

Grain samples were collected in harvest bags from each plot of the experiments to determine aflatoxin presence and concentration. Kernel samples of approximately 308 g were collected from a homogenous bulk sample and crushed using a Wiley Mill (No. 4 Grinder, Thomas Wiley Mills, Thomas Scientific, Swedesboro, NJ) fitted with a 0.05-mm sieve. Using an in-house validated Mississippi State Chemical Laboratory (MSCL) method, the grinding system was cleaned between samples using compressed air to prevent cross-contamination (A. J. Brown 2016, personal communication). From the crushed 308-g kernel mixture, 5 g of the mixture was collected, and 25 ml of a 70% methanol–water solution was mixed in a beaker by hand swirling for 3 min. The solution was filtered using grade 1 Whatman filter paper, and the liquid extraction portion was collected in 15-ml screw-top centrifuge tubes for analysis. The liquid extraction was refrigerated (4°C) until analysis but no longer than 72 h. Quantitative analysis for aflatoxin concentrations was conducted using ELISA test kits (Neogen Corp., Lexington, KY) by the Department of Biochemistry and Molecular Biology, Mississippi State, MS (Neogen 1999). Aflatoxin concentrations are expressed in parts per billion.

Data Analysis

Data for numbers of injured kernels, within each technology for treated and untreated rows, were subjected to analysis of variance procedures using PROC GLIMMIX (SAS Institute 2013). Site year (combination of year, location, and planting date) and replication nested within site year were considered random effects. Degrees of freedom were calculated using Kenwood–Roger method. Means were separated according to Fisher's protected least significant difference ($\alpha = 0.05$).

Due to variation in yield potential among site years, data for yield, test weight, and aflatoxin concentration were separated into higher and lower yield environments based on the median of the yield within each of the three technologies of the trial. This procedure was applied independently for each technology.

Data for each technology within each yield potential category were analyzed independently using regression analysis with PROC GLIMMIX (SAS Institute 2013) to estimate the impact of the number of injured kernels on yield, test weight, and aflatoxin concentration. Data for the treated and nontreated portions of each plot within each technology were combined to represent a range of injured kernels. Data for locations at which no injured kernels were observed in both the treated and nontreated portions of each plot were excluded from analysis. Outliers were determined by plotting

residual and predicted values. Based on a normal distribution, any plots with residual values more than 2 SD from the predicted value were removed from analysis; only one value in the Herculex low-yield environment was removed. Degrees of freedom were calculated using Kenwood–Roger method. Site year and replication nested within site year were designated as random effects for all analyses, allowing for inferences to be made over a range of environments (Carmer et al. 1989, Blouin et al. 2011)

Results and Discussion

Corn earworm infestations occurred in all trials (Table 2). Insect densities were on average 75% greater during the R3 growth stage compared with the R4 growth stage. A decline in insect densities from R3 to R4 was expected, as cannibalism can occur leaving only one or two later instars to successfully complete development and their migration to the soil for pupation (Mally 1892, Dial and Adler 1990). Differences in kernel damage within each technology were achieved with foliar insecticide applications directed at the ears: YieldGard ($F = 73.97$; $df = 1, 131$; $P < 0.0001$), Herculex ($F = 97.50$; $df = 1, 130$; $P < 0.0001$), and VT Triple Pro ($F = 67.15$; $df = 1, 122.8$; $P < 0.0001$) (Fig. 1).

When foliar insecticide applications were not applied, VT Triple Pro resulted in significantly lower numbers of injured kernels than the Herculex and YieldGard technologies ($F = 18.12$; $df = 2, 205$; $P < 0.0001$) (Fig. 1). These technologies were chosen for this study due to their varying levels of activity against of corn earworm.

No significant relationship between yield and injured kernels was observed in higher or lower yield environments for the Herculex, YieldGard, or VT Triple Pro technologies ($P > 0.07$ for all). Also, no significant relationship between test weight and injured kernels was observed for the Herculex, YieldGard, or VT Triple Pro technologies in the higher yield environments ($P > 0.39$ for all). In the lower yield environments, a significant relationship between test weight and injured kernels was observed for the Herculex ($F = 15.37$; $df = 1, 30.9$; $P < 0.0005$; $R^2 = 0.26$) (Fig. 2A) and the YieldGard ($F = 11.70$; $df = 1, 42.66$; $P \leq 0.0014$; $R^2 = 0.24$) (Fig. 2B) technologies. No significant relationship between test weight and injured kernels was observed for the VT Triple Pro technology ($P = 0.37$).

In the higher yield environments, a relatively weak but significant linear relationship between number of injured kernels and aflatoxin levels was observed for the Herculex ($F = 6.75$; $df = 1, 42.36$; $P = 0.01$; $R^2 = 0.25$) (Fig. 3A) and YieldGard ($F = 5.70$; $df = 1, 50.61$; $P = 0.02$; $R^2 = 0.09$) (Fig. 3B) technologies, while no significant relationship was observed for the VT Triple Pro ($P = 0.48$) technology. In the lower yields environments, no significant relationship between aflatoxin concentration and injured kernels was observed for the Herculex, YieldGard, or VT Triple Pro technologies ($P > 0.09$).

The efficacy of Bt corn technologies in reducing kernel damage from lepidopteran pests (corn earworm and fall armyworm) is well documented and ranges from moderate to very good (Storer et al. 2001, Buntin et al. 2004a, Buntin 2008, Bowen et al. 2014, Reisig et al. 2015, Reay-Jones et al. 2016). However, yield responses to corn earworm control have been variable (Buntin et al. 2004a, Buntin 2008, Reay-Jones and Wiatrak 2011, Bowen et al. 2014, Reay-Jones and Reisig 2014, Steckel and Stewart 2015). In some studies, a yield increase was observed with kernel damage reduction from the use of Bt corn (DeLamar et al. 1999a, b, c, d, e; Buntin et al. 2004a), while in others, no impact on yield was observed (Buntin et al. 2004a, Buntin 2008, Reay-Jones and Wiatrak 2011, Bowen et al. 2014, Reay-Jones and Reisig 2014, Steckel and Stewart 2015). In the current study, natural corn infestations did not cause enough kernel

Table 2. Average number of corn earworm larva per ear present at milk (R3) and dough (R4) growth stage at each location over all plant dates

Year	Location	Technology (hybrid)	Small	R3, Med	Large	Small	R4, Med	Large
2011	Starkville, MS	Herculex	0.1	0.3	0.4	0.0	0.1	0.1
2011	Starkville, MS	YieldGard	0.1	0.3	0.4	0.0	0.0	0.1
2011	Starkville, MS	VT Triple Pro	0.2	0.3	0.4	0.0	0.1	0.1
2012	Starkville, MS	Herculex	0.7	0.6	0.4	0.1	0.2	0.2
2012	Starkville, MS	YieldGard	0.8	0.6	0.3	0.2	0.2	0.3
2012	Starkville, MS	VT Triple Pro	0.6	0.5	0.5	0.2	0.2	0.3
2011	Stoneville, MS	Herculex	1.3	0.6	0.3	0.4	0.3	0.3
2011	Stoneville, MS	YieldGard	0.8	0.7	0.4	0.3	0.2	0.2
2011	Stoneville, MS	VT Triple Pro	1.1	0.3	0.1	0.4	0.3	0.1
2012	Stoneville, MS	Herculex	0.5	1.3	0.3	0.0	0.1	0.1
2012	Stoneville, MS	YieldGard	0.2	0.7	0.3	0.0	0.0	0.0
2012	Stoneville, MS	VT Triple Pro	0.6	0.5	0.1	0.1	0.1	0.1
2011	Jackson, TN	Herculex	0.9	1.2	1.0	0.1	0.2	0.4
2011	Jackson, TN	YieldGard	1.0	1.1	0.9	0.0	0.2	0.3
2011	Jackson, TN	VT Triple Pro	1.2	0.3	0.1	0.1	0.2	0.3
2012	Jackson, TN	Herculex	1.6	0.4	0.2	0.1	0.3	0.1
2012	Jackson, TN	YieldGard	1.8	0.3	0.0	0.1	0.2	0.3
2012	Jackson, TN	VT Triple Pro	0.4	0.0	0.0	0.1	0.2	0.2
2011	Griffin, GA	Herculex	1.1	0.6	0.1	0.4	0.2	0.1
2011	Griffin, GA	YieldGard	1.8	0.6	0.2	0.2	0.3	0.1
2011	Griffin, GA	VT Triple Pro	0.9	0.2	0.0	0.5	0.2	0.1
2012	Griffin, GA	Herculex	1.1	0.4	0.1	0.1	0.1	0.4
2012	Griffin, GA	YieldGard	0.8	0.6	0.3	0.1	0.3	0.3
2012	Griffin, GA	VT Triple Pro	0.5	0.1	0.0	0.3	0.2	0.1

Size classification of larva based on length: (small: <0.64 cm [0.25 inch]; Medium: 0.64 to 1.27 cm [0.25 to 0.5 inch]; Large: >1.27 cm [0.5 inch]). Data were collected from rows not treated with insecticide. Infestation data not presented for LA location.

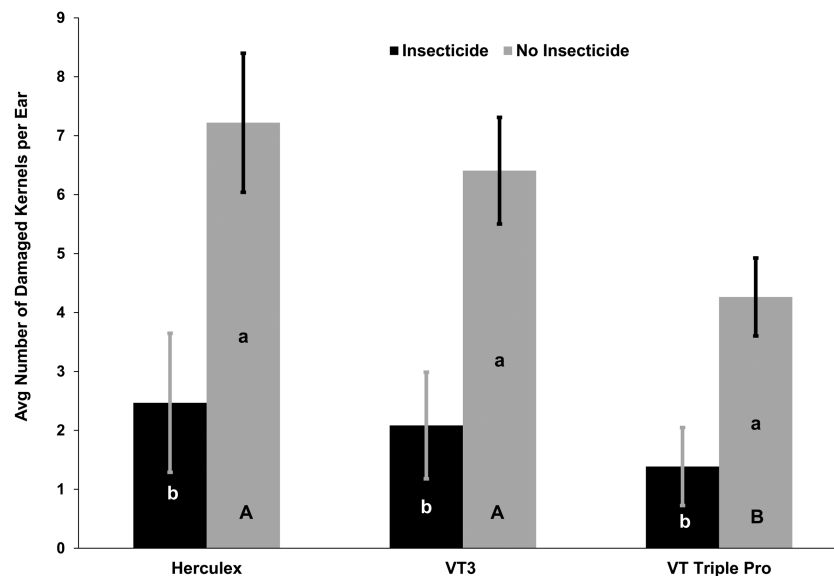


Fig. 1. Mean number of corn earworm injured kernels (Mean \pm SEM) for corn hybrids expressing the Herculex (Cry1F), YieldGard (Cry1Ab), or Genuity VT Triple Pro (Cry1A.105 + Cry2Ab2 + Cry3Bb1 [corn rootworm activity only]) technologies. Capital letters represent analysis for mean number of injured kernels for corn hybrids that did not receive supplemental insecticide applications. Lowercase letters represent analysis for comparing the mean number of injured kernels where a supplemental insecticide application was received to mean number of injured kernels where no supplemental insecticide application was received, within each technology. Bars within a technology with a common letter are not significantly different ($P \geq 0.05$, Fisher's PLSD).

damage to impact yield. There were also no significant differences in test weight between Bt and non-Bt hybrids where corn earworm kernel damage was reduced (Buntin et al. 2004b, Buntin 2008). In the current study, no significant relationship between kernel damage and yield or test weight was observed, with two exceptions for test weight.

There are several possibilities as to why a reduction in corn earworm feeding on kernels may not result in significant yield increases. Corn earworm normally feeds on kernels near the tip of the ear (Klostermeyer and Rasmussen 1953, Steffey et al. 1999, Bohnenblust et al. 2013), and many of the kernels near the ear tip will not develop into mature kernels that contribute to yield (Nishikawa and Kudo

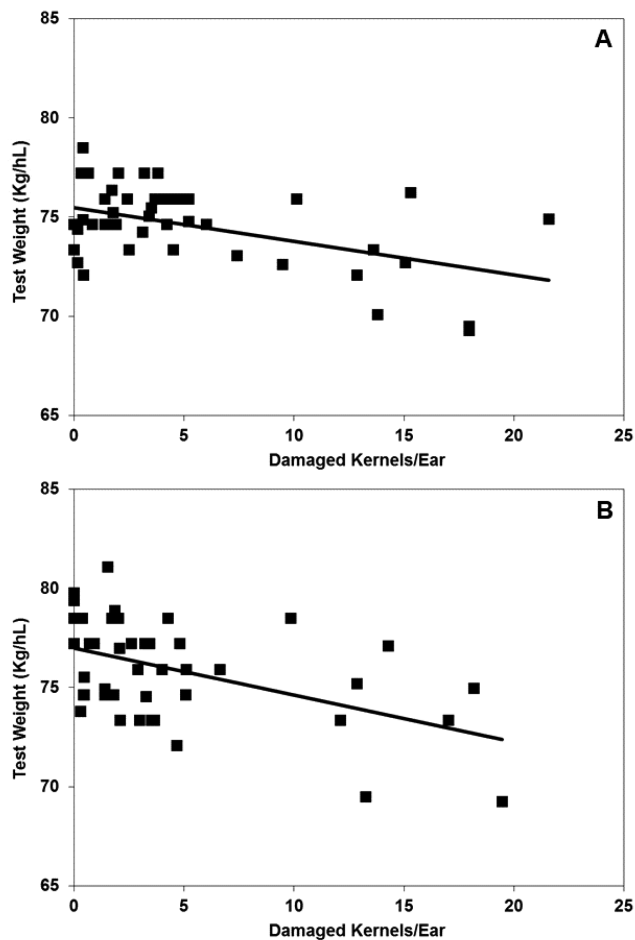


Fig. 2. Relationship between numbers of injured kernels per ear and test weight for a corn hybrid in lower yield environments expressing the (A) Herculex (Cry1F) technology ($\text{kg/hl} = 75.481 - 0.1703 \times [0.04422]$; $P < 0.0005$; $R^2 = 0.26$) and the (B) YieldGard (Cry1Ab) technology ($\text{kg/hl} = 76.987 - 0.236 \times [0.06879]$; $P < 0.0014$; $R^2 = 0.24$).

1973). It is also possible that kernels further down the ear may compensate for damage occurring near the ear tip (Dyer 1975, Woronecki et al. 1980, White and Scott 1983, Steckel and Stewart 2015). Another possibility is that corn earworm infestations do not cause sufficient damage to impact yield in most cases (Bowen et al. 2014, Reay-Jones and Reisig 2014, Steckel and Stewart 2015). Also, efficiency of current harvest machinery may influence the ability to detect yield losses from corn earworm infestations. The kernels near the ear tip generally have a lower weight than those further down the ear (Tollenaar and Daynard 1978), which would increase the potential of those kernels being expelled with cobs and stalk material during mechanical harvest. Many of the published studies utilized mechanical harvest methods which can be very inefficient and mask small but positive differences in grain yield (Buntin et al. 2004a, Buntin 2008, Bowen et al. 2014, Reay-Jones and Reisig 2014). Surveys of harvested commercial corn fields in Mississippi reported up to 143,000 volunteer corn plants per ha (Babu et al. 2014), the result of mature grain (seeds) being discharged back to the field during the mechanical harvesting process. These densities are greater than 1.5 times the recommended seeding rate for corn (McClure 2010, Lee 2011, Larson 2012, Anonymous 2016). Many of the factors mentioned above may contribute to the lack of yield responses to kernel damage reductions. Improved harvest efficiency

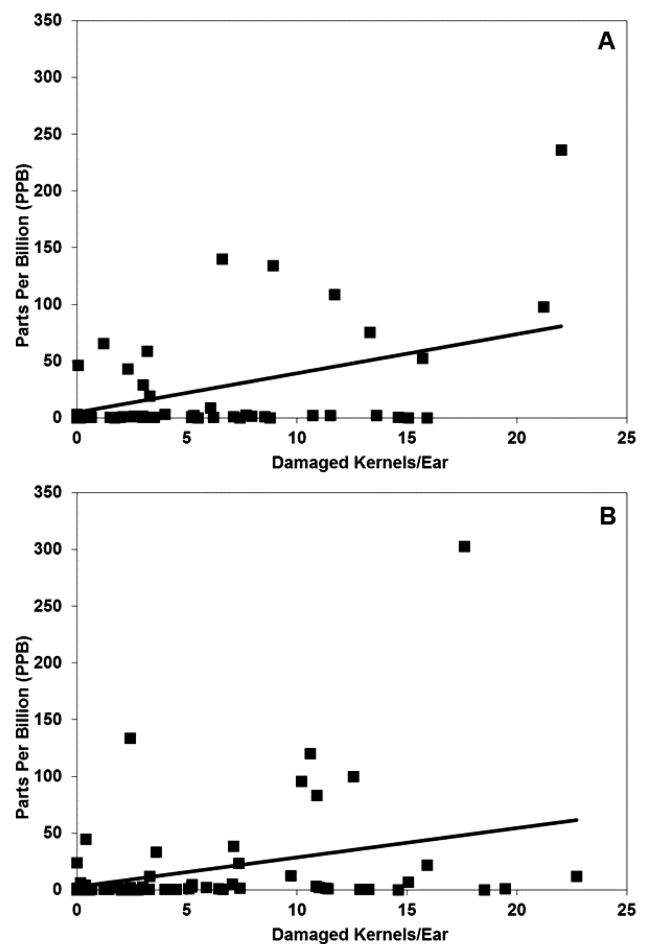


Fig. 3. Relationship between numbers of injured kernels per ear and aflatoxin concentration for a corn hybrid in higher yield environments expressing the (A) Herculex (Cry1F) technology ($\text{ppb} = 3.4474 \times [1.0504] + 4.9244$; $P = 0.01$; $R^2 = 0.25$) and the (B) YieldGard (Cry1Ab) technology ($\text{ppb} = 2.5679 \times [1.0676] + 3.0615$; $P = 0.02$; $R^2 = 0.09$).

may or may not result in positive responses to kernel damage reduction but would result in higher harvest yields for growers.

Lepidopteran larvae do not appear to directly transport fungus into the ear; however, larval feeding can provide a pathway of fungal entry (Widstrom et al. 1975). It appears that any impacts on aflatoxin concentrations associated with Bt corn hybrids are through reductions in insect injury, which reduces avenues for fungal infection and not from direct action of the Bt traits on the fungus (Dowd 2000, Williams et al. 2002). The efficacy of transgenic Bt corn technologies against lepidopteran larvae is well documented. However, reductions in kernel damage have not consistently translated into reduced aflatoxin concentrations (Odvody et al. 2000, Buntin et al. 2001, Munkvold 2003, Buntin et al. 2004a, Wu et al. 2004, Wu 2007). Injury to plant reproductive parts caused by insects can increase the risk of mycotoxin contamination which can generally occur in uninjured grain but is more likely to occur in injured grain (Diener et al. 1987, Niu et al. 2009). In the current study, a significant relationship between kernel damage and aflatoxin concentration was observed with two technologies. Insect injury, such as that caused by lepidopteran and coleopteran insects, is not a primary factor that influences aflatoxin production (Widstrom et al. 1975, Odvody et al. 2000, Buntin et al. 2001, Wu et al. 2004, Wu 2007, Ni et al. 2011), but the presence of grain injury may contribute to an

existing *Aspergillus* spp. infection. Other plant stress factors such as drought and nutrient deficiencies have a much greater influence on the incidence and overall aflatoxin production (Jones et al. 1981, Dorner et al. 1999). Based on these data, the economic value of pyramided Bt corn traits to corn producers appears to be from protection against other lepidopteran insect pests including European and southwestern corn borer and fall armyworm in southern geographies, and as a resistance management tool to maintain the durability of the technologies.

Acknowledgments

We thank the Mississippi Corn Promotion Board and Monsanto for providing financial support of this study. The Mississippi Agricultural and Forestry Experiment Station (B. Garrard), Mississippi State Extension Service, Mississippi State University Delta Research and Extension Center (N. Wright), University of Tennessee West Tennessee Experiment Station (S. Steckel), LSU AgCenter's Macon Ridge Research Station (S. Williams and K. Emfinger), University of Georgia Griffin Experiment Station (W.R. Slaughter and J.T. Strickland), and Mississippi State Chemical Laboratory (A. Brown, G. Hagood, B. Davis, and J. Rodriguez) personnel for all their support and supplement of space and resources while conducting this study.

References Cited

- Anonymous. 2016. Corn Hybrids for Grain 2016. Louisiana State University Extension Publ. 2827. Louisiana State University, Baton Rouge, LA.
- Babu, A., D. R. Cook, M. A. Caprio, K. C. Allen, and F. R. Musser. 2014. Prevalence of *Helicoverpa zea* (Lepidoptera: Noctuidae) on late season volunteer corn in Mississippi: implications on Bt resistance management. *Crop Prot.* 64: 207–214.
- Bennett, J. W., and M. Klich. 2003. Mycotoxins. *Clin. Microbiol. Rev.* 16: 497–516.
- Blouin, D. C., E. P. Webster, and J. A. Bond. 2011. On the analysis of combined experiments. *Weed Technol.* 25: 165–169.
- Bohnenblust, E., J. Breining, S. Fleischer, G. Roth, and J. Tooker. 2013. Corn earworm (Lepidoptera: Noctuidae) in northeastern field corn: infestation levels and the value of transgenic hybrids. *J. Econ. Entomol.* 106: 1250–1259.
- Bowen, K. L., K. L. Flanders, A. K. Hagan, and B. Ortiz. 2014. Insect damage, aflatoxin content, and yield of Bt corn in Alabama. *J. Econ. Entomol.* 107: 1818–1827.
- 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.
- Buntin, G. D., R. D. Lee, D. M. Wilson, and R. M. McPherson. 2001. Evaluation of YieldGard® transgenic resistance for control of fall armyworm and corn earworm (Lepidoptera: Noctuidae) on corn. *Fla. Entomol.* 84: 37–42.
- Buntin, G. D., J. N. All, R. D. Lee, and D. M. Wilson. 2004a. Plant-incorporated *Bacillus thuringiensis* resistance for control of fall armyworm and corn earworm (Lepidoptera: Noctuidae) in corn. *J. Econ. Entomol.* 97: 1603–1611.
- Buntin, G. D., K. L. Flanders, and R. E. Lynch. 2004b. Assessment of experimental Bt events against fall armyworm and corn earworm in field corn. *J. Econ. Entomol.* 97: 259–264.
- Carmer, S. G., W. E. Nyquist, and W. M. Walker. 1989. Least significant differences for combined analyses of experiments with two- or three- factor treatment designs. *Agron. J.* 81: 665–672.
- Charmley, L. L., H. L. Trenholm, D. B. Prelusky, and A. Rosenberg. 1995. Economic losses and decontamination. *Nat. Toxins.* 3: 199–203.
- DeLamar, Z. D., K. L. Flanders, J. H. Holliman, and P. L. Mask. 1999a. Efficacy of transgenic corn against southern insect pests in Marion Junction, Alabama, 1998. *Arthropod Manag. Tests.* 24: 417–418.
- DeLamar, Z. D., K. L. Flanders, S. P. Nightengale, and P. L. Mask. 1999b. Efficacy of transgenic corn against southern insect pests in Tallassee, Alabama, 1998. *Arthropod Manag. Tests.* 24: 418–419.
- DeLamar, Z. D., K. L. Flanders, R. A. Dawkins, and P. L. Mask. 1999c. Efficacy of transgenic corn against southern insect pests in Crossville, Alabama, 1998. *Arthropod Manag. Tests.* 24: 420–421.
- DeLamar, Z. D., K. L. Flanders, M. D. Pegues, and P. L. Mask. 1999d. Efficacy of transgenic corn against southern insect pests in Fairhope, Alabama, 1998. *Arthropod Manag. Tests.* 24: 421–423.
- DeLamar, Z. D., K. L. Flanders, L. W. Wells, and P. L. Mask. 1999e. Efficacy of transgenic corn against southern insect pests in Headland, Alabama, 1998. *Arthropod Manag. Tests.* 24: 423–424.
- DePew, L. J. 1966. Evaluation of insecticides for corn earworm control on sweet corn. *J. Econ. Entomol.* 59: 518–520.
- Dial, C. I., and P. H. Adler. 1990. Larval behavior and cannibalism in *Heliothis zea* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 83: 258–263.
- Diener, U. L., R. J. Cole, T. H. Sanders, G. A. Payne, L. S. Lee, and M. A. Klich. 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Annu. Rev. Phytopathol.* 25: 249–270.
- Dorner, J. W., R. J. Cole, and D. T. Wicklow. 1999. Aflatoxin reduction in corn through field application of competitive fungi. *J. Food Prot.* 62: 650–656.
- Dowd, P. F. 2000. Indirect reduction of ear molds and associated mycotoxins in *Bacillus thuringiensis* corn under controlled and open field conditions: utility and limitations. *J. Econ. Entomol.* 93: 1669–1679.
- Dyer, M. I. 1975. The effects of red-winged blackbirds (*Agelaius phoeniceus* L.) on biomass production of corn grains (*Zea mays* L.). *J. Appl. Ecol.* 12: 719–726.
- Foster, R. E. 1989. Strategies for protecting sweet corn ears from damage by fall armyworm (Lepidoptera: Noctuidae) in southern Florida. *Fla. Entomol.* 72: 146–151.
- Gryspeirt, A., and J. C. Grégoire. 2012. Effectiveness of the high dose/refuge strategy for managing pest resistance to *Bacillus thuringiensis* (Bt) plants expressing one or two toxins. *Toxins (Basel)*. 4: 810–835.
- Hardke, J. T., B. R. Leonard, F. Huang, and R. E. Jackson. 2011. Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) on transgenic field corn expressing *Bacillus thuringiensis* Cry proteins. *Crop Prot.* 30: 168–172.
- Hedayati, M. T., A. C. Pasqualotto, P. A. Warn, P. Bowyer, and D. W. Denning. 2007. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiology*. 153: 1677–1692.
- Hesseltine, C. W., O. L. Shotwell, J. J. Ellis, and R. D. Stubblefield. 1966. Aflatoxin formation by *Aspergillus flavus*. *Bacteriol. Rev.* 30: 795–805.
- Huang, F., B. R. Leonard, and J. Baldwin. 2006a. Managing Corn Borers with Transgenic Bt Corn Technology in Louisiana, vol. 49. LSU AgCenter, Baton Rouge, LA. 25–26.
- Huang, F., B. R. Leonard, D. R. Cook, D. R. Lee, D. A. Andow, J. L. Baldwin, K. V. Tindall, and X. Wu. 2006b. Frequency of alleles conferring resistance to *Bacillus thuringiensis* maize in Louisiana populations of southwestern corn (Lepidoptera: Crambidae). *Entomol. Exp. Appl.* 122: 53–58.
- Huang, F., J. A. Qureshi, R. L. Meagher Jr, D. D. Reisig, G. P. Head, D. A. Andow, X. Ni, D. Kerns, G. D. Buntin, Y. Niu, et al. 2014. Cry1F resistance in fall armyworm *Spodoptera frugiperda*: single gene versus pyramided Bt maize. *PLoS One*. 9: e112958.
- Janes, M. J., and G. L. Greene. 1969. Control of fall armyworms and corn earworms on sweet corn ears in central and south Florida. *J. Econ. Entomol.* 62: 1031–1033.
- Jones, R. K., H. E. Duncan, and P. B. Hamilton. 1981. Planting date, harvest date, and irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn. *Phytopathology* 71: 810.
- Klostermeyer, E. C., and W. B. Rasmussen. 1953. The effect of soil insecticide treatments on mite population and damage. *J. Econ. Entomol.* 46: 910–912.
- Koenning, S., and G. Payne. 1999. Mycotoxins in Corn. Plant Pathology Extension Information Note. CORN001. College of Agricultural and Life Sciences, North Carolina State University.
- Larson, E. 2012. How to plant corn for higher yields. mississippi-crops.com/2012/03/19/how-to-plant-corn-for-higher-yields-2/.
- Lee, R. D. 2011. Planting Guide for Row Crops in Georgia. University of Georgia Extension Circular. 813. University of Georgia, Athens, GA.
- Lee, R. D. 2013. A Guide to Corn Production in Georgia in 2013. University of Georgia Extension Misc. Publication. CSS 01-2012. University of Georgia, Athens, GA.

- Luo, K., D. Banks, and M. J. Adang. 1999. Toxicity, binding, and permeability analyses of four *Bacillus thuringiensis* Cry1 delta-endotoxins using brush border membrane vesicles of *Spodoptera exigua* and *Spodoptera frugiperda*. *Appl. Environ. Microbiol.* 65: 457–464.
- Mally, F. W. 1892. Report of progress in the investigation of the cotton bollworm. U.S. Dep. Agric., Div. Entomol. Bull. 26: 45–56.
- McClure, A. T. 2010. Planting Corn in Tennessee. University of Tennessee Extension Publ. W077. University of Tennessee, Knoxville, TN.
- Munkvold, G. P. 2003. Cultural and genetic approaches to managing mycotoxins in maize. *Annu. Rev. Phytopathol.* 41: 99–116.
- Neogen. 1999. Veratox Instruction Manual #8030. Neogen Corp, Miami, FL.
- Ni, X., J. P. Wilson, G. D. Buntin, B. Guo, M. D. Krakowsky, R. D. Lee, T. E. Cottrell, B. T. Scully, A. Huffaker, and E. A. Schmelz. 2011. Spatial patterns of aflatoxin levels in relation to ear-feeding insect damage in pre-harvest corn. *Toxins (Basel)*. 3: 920–931.
- Nishikawa, H., and M. Kudo. 1973. Explicational studies on the sterile ear as appeared on mechanized cultivation of the corn plant (*Zea mays* L.). [In Japanese, English summary]. *Tohoku Agric. Exp. Sta. Res. Rep.* 44: 51–95.
- Niu, G., J. Siegel, M. A. Schuler, and M. R. Berenbaum. 2009. Comparative toxicity of mycotoxins to navel orangeworm (*Amyelois transitella*) and corn earworm (*Helicoverpa zea*). *J. Chem. Ecol.* 35: 951–957.
- Odvody, G. N., C. F. Chilcutt, R. D. Parker, and J. H. Benedict. 2000. Aflatoxin and insect response of near-isogenic Bt and non-Bt commercial corn hybrids in South Texas. *Proc. USDA-ARS Aflatoxin/Fumonisin Workshop, Fish Camp, CA*, Oct. 25–27, p. 121.
- Ostlie, K., W. D. Hutchison, and R. L. Hellmich. 1997. Bt Corn and European Corn Borer: Long-term Success Through Resistance Management. Cooperative Extension Service. Extension Publication 602. University of Minnesota, St. Paul, MN.
- Reay-Jones, F. P. F., and D. D. Reising. 2014. Impact of corn earworm injury on yield of transgenic corn producing Bt toxins in the Carolinas. *J. Econ. Entomol.* 107: 1001–1009.
- Reay-Jones, F., and P. Wiatrak. 2011. Evaluation of new transgenic corn hybrids producing multiple *Bacillus thuringiensis* toxins in South Carolina. *J. Entomol. Sci.* 46:152–164.
- Reay-Jones, F. P., R. T. Bessin, M. J. Brewer, D. G. Buntin, A. L. Catchot, D. R. Cook, K. L. Flanders, D. L. Kerns, R. P. Porter, D. D. Reising, et al. 2016. Impact of lepidoptera (Crambidae, Noctuidae, and Pyralidae) pests on corn containing pyramided Bt traits and a blended refuge in the southern United States. *J. Econ. Entomol.* 109: 1859–1871.
- Reising, D. D., D. S. Akin, J. N. All, R. T. Bessin, M. J. Brewer, D. G. Buntin, A. L. Catchot, D. Cook, K. L. Flanders, F. N. Huang, et al. 2015. Lepidoptera (Crambidae, Noctuidae, and Pyralidae) injury to corn containing single and pyramided Bt traits, and blended or block refuge, in the southern United States. *J. Econ. Entomol.* 108: 157–165.
- SAS Institute. 2013. The SAS System for Windows. Release 9.3. SAS Institute, Cary, NC.
- Shelton, A. M., D. L. Olmstead, E. C. Burkness, W. D. Hutchison, G. Dively, C. Welty, and A. N. Sparks. 2013. Multi-state trials of Bt sweet corn varieties for control of the corn earworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 106: 2151–2159.
- Shelton, A. M., J. Z. Zhao, and R. T. Roush. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. *Annu. Rev. Entomol.* 47: 845–881.
- Siebert, M., J. M. Babcock, S. Nolting, A. C. Santos, J. J. Adamczyk, P. A. Neese, J. E. King, J. N. Jenkins, J. McCarty, G. M. Lorenz, et al. 2008a. Efficacy of Cry1F insecticidal protein in maize and cotton for control of fall armyworm (Lepidoptera: Noctuidae). *Fla. Entomol.* 91: 555–565.
- Siebert, M. W., K. V. Tindall, B. R. Leonard, J. W. Van Duyn, and J. M. Babcock. 2008b. Evaluation of corn hybrids expressing Cry1F (Herculex I® insect protection) against fall armyworm (Lepidoptera: Noctuidae) in southern United States. *J. Entomol. Sci.* 43: 41–51.
- Steckel, S., and S. D. Stewart. 2015. Intraear compensation of field corn, *Zea mays*, from simulated and naturally occurring injury by ear-feeding larvae. *J. Econ. Entomol.* 108: 1106–1114.
- Steffey, K. L., M. E. Rice, J. All, D. A. Andow, M. E. Gray, and J. W. Van Duyn. 1999. Handbook of Corn Insects. Entomological Society of America, Lanham, MD.
- 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.
- Tabashnik, B. E., T. Brévault, and Y. Carrière. 2013. Insect resistance to Bt crops: lessons from the first billion acres. *Nat. Biotechnol.* 31: 510.
- Tollenaar, M., and T. B. Daynard. 1978. Kernel growth and development at two positions on the ear of maize (*Zea mays*). *Can. J. Plant Sci.* 58: 189–197.
- USDA NASS. 2012. NASS Data and Statistics. United States Department of Agriculture National Agricultural Statistics Service. http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp.
- White, S. M., and D. R. Scott. 1983. Corn earworm (Lepidoptera: Noctuidae) in Idaho: corn kernel compensation for larval damage. *J. Econ. Entomol.* 76: 1374–1376.
- Widstrom, N. W., A. N. Sparks, E. B. Lillehoj, and W. F. Kwolek. 1975. Aflatoxin production and lepidopteran insect injury on corn in Georgia. *J. Econ. Entomol.* 68: 855–856.
- Williams, W. P., G. L. Windham, P. M. Buckley, and C. A. Daves. 2002. Aflatoxin accumulation in conventional and transgenic corn hybrids infested with southwestern corn borer (Lepidoptera: Crambidae). *J. Agric. Urban Entomol.* 19: 227–236.
- Woronecki, P. P., R. A. Stehn, and R. A. Dolbeer. 1980. Compensatory response of maturing corn kernels following simulated damage by birds. *J. Appl. Ecol.* 17: 737–746.
- Wu, F. 2006. Mycotoxin reduction in Bt corn: potential economic, health, and regulatory impacts. *Transgenic Res.* 15: 277–289.
- Wu, F. 2007. Bt corn and impact on mycotoxins. *CAB Rev.: Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* 2: 1–8.
- Wu, F., J. D. Miller, and E. A. Casman. 2004. The economic impact of Bt corn resulting from mycotoxin reduction. *Toxin Rev.* 23: 397–424.

Reproduced with permission of copyright owner. Further reproduction prohibited without permission.