

## EFFICACY OF CRY1F INSECTICIDAL PROTEIN IN MAIZE AND COTTON FOR CONTROL OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE)

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## ABSTRACT

Efficacy of maize, *Zea mays* L., hybrids and cotton, *Gossypium hirsutum* (L.), varieties expressing Cry1F insecticidal crystal protein of *Bacillus thuringiensis* (Bt) var. *aizawai* Berliner (transformation event TC1507 in corn and event DAS-24236-5 in cotton) was evaluated for control of fall armyworm, *Spodoptera frugiperda* (J.E. Smith). Control of natural and artificial fall armyworm infestations of eggs and various larval stages to 3 Cry1F and non-Bt maize isoline pairs at V4-V7 corn growth stage was evaluated at 10 locations across the United States and Brazil. Varieties producing the Cry1F protein provided high levels of control. Furthermore, control provided by Cry1F-maize hybrids was frequently better than when fall armyworm were managed with 3 applications of foliar insecticides. Efficacy of transgenic Cry1Ac:Cry1F cotton against fall armyworm was evaluated for 5 varieties during anthesis in laboratory and natural infestation field studies in the southern United States. Laboratory colonies of fall armyworm originally collected from corn, bermudagrass, *Cynodon dactylon* (L.), cotton, and royal paulownia, *Paulownia tomentosa* (Thunb.) and determined to be either the rice or corn-associated host strain, all resulted in low levels of survival when fed matures leaves of Cry1Ac:Cry1F-cotton. In natural infestation studies, levels of fall armyworm in squares (flower buds), flowers, and bolls (fruit), were significantly lower in cotton containing Cry1F as compared to non-Bt cotton. These results demonstrate that maize hybrids and cotton varieties containing Cry1F can be an important component in an overall management program for fall armyworm across a broad range of geographies and crops.

Key Words: *Bacillus thuringiensis* var. *aizawai*, *Spodoptera frugiperda*, cotton, maize, IPM, Cry1F

## RESUMEN

La eficacia de los híbridos de maíz, *Zea mays* L. y de las variedades de algodón, *Gossypium hirsutum* (L.), en expresar la proteína cristalizada insecticida Cry1F de *Bacillus thuringiensis* (Bt) var. *aizawai* Berliner (transformación evento TC1507 en maíz y evento DAS-24236-5 en algodón) fue evaluada para el control del gusano cogollero. El control de infestaciones naturales y artificiales de huevos y varios estadios de larvas del gusano cogollero en 3 pares de isolíneas de Cry1F y de maíz sin Bt en maíz en las etapas V4-V7 del desarrollo, fueron evaluadas en 10 localidades a través de los Estados Unidos y Brasil. Las variedades que producen la proteína Cry1F proveyeron altos niveles de control. Además, el control proveído por los híbridos Cry1F de maíz frecuentemente fue mejor que cuando se aplicaron 3 insecticidas foliares para el manejo del gusano cogollero. La eficacia de algodón transgénico Cry1Ac:Cry1F contra el gusano cogollero fue evaluada en 5 variedades durante la antesis en los estudios de laboratorio y en las infestaciones naturales en el campo en el sur de los Estados Unidos. Las colonias del gusano cogollero en el laboratorio que fueron recolectadas originalmente sobre maíz; el pasto bermuda, *Cynodon dactylon* (L.); el algodón; y la paulownia imperial, *Paulownia tomentosa* (Thunb.) y que fueran determinadas como la sepa asociada con arroz o maíz, todas resultaron en tener niveles de sobrevivencia mas bajos cuando las alimentaron de hojas maduras del algodón con Cry1Ac:Cry1F. En estudios de infestaciones naturales, los niveles de los gusanos cogolleros en las brácteas, flores y bellotas (fruta), fueron significativamente más bajos en algodón que tenía Cry1F en comparación a algodón sin

Bt. Estos resultados demuestran que los híbridos de maíz y variedades de algodón que contienen Cry1F pueden ser un componente importante en un programa total del manejo de gusano cogollero a través de un amplio rango geográfico y rango de hospederos.

Transformation of maize, *Zea mays* L., and cotton, *Gossypium hirsutum* (L.), to express *Bacillus thuringiensis* insecticidal toxins has resulted in numerous benefits to producers and agroecosystems. Transgenic crops provide the opportunity to control insecticide-resistant pest species, maximize crop yields, conserve beneficial arthropods, and reduce the frequency of applications of synthetic insecticides (Edge et al. 2001; Shelton et al. 2002). Additionally, transgenic technologies can offer control of insect pests generally difficult to manage with synthetic insecticides. One example of these benefits is illustrated in the management of fall armyworm, *Spodoptera frugiperda* (J. E. Smith), with transgenic Bt technology. Biological characteristics of fall armyworm, coupled with operational factors that result in suboptimal control with insecticides, make transgenic options attractive for management in maize and cotton.

Fall armyworm can be a destructive pest of cotton and maize produced in the U.S., Central America, and South America (Sparks 1979; Sena et al. 2003). In maize, fall armyworms are capable of causing defoliation during the whorl stage as well as direct injury to the ear (Labatte 1993; Davis et al. 1998). Although fall armyworm may injure maize plants in nearly all stages of development, infestations in the U.S. concentrate on plants that are in whorl stages, particularly in later-planted maize where these vegetative stages are synchronized with high moth abundance (Quisenberry 1999). Although considered an occasional pest in the U.S., fall armyworm is a primary pest in tropical climates. Factors that contribute to the elevated pest status of fall armyworm in those locations include continuous production of maize, multiple generations of intense infestations, and widespread insecticide resistance (Cruz 1995). In Brazil, the potential for yield losses in maize has ranged from 17 to 38.7% (Fernandes et al. 2003). Historical reports of significant yield loss can also be found in Mexico, Central America, and Argentina (Perdiguero et al. 1967; Andrews 1980).

Fall armyworms can be a destructive pest in cotton. As with maize, fall armyworm is considered an occasional pest of cotton in the U.S. and a primary pest of cotton in Brazil (Santos et al. 2005). Moth oviposition and subsequent feeding by first and second instar larvae commonly occur on leaves within the lower two-thirds of the plant canopy (Ali et al. 1990). Later instars predominantly feed on fruiting structures including squares (flower buds), flowers, and bolls (fruit) (Ali et al. 1990).

The spatial distribution of fall armyworm eggs and small larvae in maize and cotton make detection and control with foliar insecticides challenging. Insecticide spray coverage in maize is difficult because larvae are located in the wrapped-up leaves of the whorl within 1 d of egg eclosion until larval development is complete (Labatte 1993). Similarly in cotton, insecticide coverage is inadequate for larvae located in lower portions of the plant canopy and for larger larvae concealed in fruiting structures. Failure to control fall armyworm as early instar larvae may be problematic because fall armyworm become tolerant to insecticides as they increase in size (Yu 1983; Mink & Luttrell 1989). Selection of an effective insecticide, timing and method of application, and reinfestation are additional considerations that affect management of fall armyworm in maize and cotton (Cook et al. 2004; Ghidiu & Andaloro 1993). Transgenic plants that express Bt proteins throughout various tissue types and for the duration of plant development are useful for overcoming many of the limitations associated with managing fall armyworm with synthetic foliar insecticides.

Single-gene Bt maize hybrids and cotton varieties became available to producers during 1996 and contained Cry1Ab (events MON 810, Bt-176, and Bt-11) and Cry1Ac (event MON 531), respectively. The rationale for introducing these transgenic technologies was to control pests of global and economic importance including European corn borer, *Ostrinia nubilalis* (Hübner), and southwestern corn borer, *Diatraea grandiosella* Dyar, in maize and heliothines tobacco budworm, *Heliothis virescens* (F.) and bollworm, *Helicoverpa zea* (Boddie) and pink bollworm, *Pectinophora gossypiella* (Saunders), in cotton (Milfin 1996; Gianessi et al. 2002a; Gianessi et al. 2002b). Bt maize hybrids containing Cry1Ab protein provide excellent control of European corn borer and southwestern corn borer (Archer et al. 2001; Castro et al. 2004). However, larval establishment can occur on Cry1Ab maize hybrids for other species that feed on foliage and ears including fall armyworm, black cutworm, *Agrotis ipsilon* (Hufnagel), western bean cutworm, *Richia albicosta* (Smith), and corn earworm, *Helicoverpa zea* (Boddie) (Pilcher et al. 1997; Buntin et al. 2001; Catangui & Berg 2006).

Single-gene Bt cotton varieties contain Cry1Ac protein and provide absolute control of tobacco budworm and pink bollworm (MacIntosh et al. 1990; Tabashnik et al. 2000; Jackson et al. 2003). However, as with maize, it was recognized that a single Bt protein could not provide broad spec-

trum lepidopteran control. Thus, bollworm and other secondary lepidopteran pests infesting single-gene Bt cotton would need to be managed with supplemental insecticides to prevent economic losses (Gore et al. 2001; Stewart et al. 2001). Therefore, new Bt proteins can broaden the spectrum of lepidopteran activity in both Bt maize hybrids and Bt cotton varieties. Combinations of insect resistant traits also aid in resistance management of target Lepidoptera pest species (McGaughey & Whalon 1992; Tabashnik 1994; Gould 1998; Stewart et al. 2001).

Maize hybrids that express the Cry1F insecticidal crystal protein of *B. thuringiensis* var. *aizawai* were commercialized in the U.S. in 2003 (event TC1507, Herculex® I Insect Protection). Cry1F maize hybrids provide control of not only European corn borer and southwestern corn borer, but also provide protection against damaging infestations of other lepidopteran pests including sugarcane borer, *Diatraea saccharalis* (F.), fall armyworm, black cutworm, and western bean cutworm (U.S. Environmental Protection Agency 2005; Catangui & Berg 2006; Siebert et al. 2008). The first dual-toxin Bt cotton varieties were available during the 2003 growing season and contained Cry1Ac and Cry2Ab (event MON 15985). Closely thereafter in 2005, varieties containing combined Cry1Ac (event DAS-21Ø23-5) and Cry1F (event DAS-24236-5) (WideStrike™, Dow AgroSciences LLC, Indianapolis, IN) also became available to producers. The addition of either Cry1F or Cry2Ab to Cry1Ac has allowed for improved control of secondary pests (Stewart et al. 2001; Willrich et al. 2005; Greenberg & Adamczyk 2007).

The objective of the following series of experiments was to compare the efficacy of maize hybrids and cotton varieties containing transgenic Cry1F for control of fall armyworm. Cry1F efficacy was compared across numerous geographies and plant tissue types.

## MATERIALS AND METHODS

### Laboratory and Field Studies on Maize Producing the Cry1F Bt Protein

Studies evaluating fall armyworm injury to vegetative (whorl) stage Cry1F and non-Bt maize hybrids were conducted in 3 locations (7 studies) in the U.S. from 2002-2006 and in 3 locations in Brazil during 2007 (Table 1). At each location, Mycogen corn hybrids (Mycogen Seeds, LLC, Indianapolis, IN) producing Cry1F were compared to non-Bt near-isolines. Up to 3 Cry1F/non-Bt pairs were evaluated at a single location (Table 1). In Brazil, an additional treatment of the non-Bt corn hybrid, managed with synthetic insecticides targeting fall armyworm, was included for comparison. In those studies, methomyl (Lannate 216 g/L

SL, DuPont Crop Protection, Alphaville, SP, Brazil),  $\lambda$ -cyhalothrin (Karate Zeon 50 g/L CS, Syngenta Crop Protection, Inc., Santo Amaro, SP, Brazil), and lufenuron (Match 50 g/L EC, Syngenta Crop Protection, Inc., Santo Amaro, SP, Brazil) were applied sequentially at a 4-7 d interval between applications beginning at the initiation of natural infestations. Treatments in the field were planted in a randomized complete block design with 4 replications at all locations. Plot size across locations ranged from 2 to 8 rows (76.2 to 101.6-cm row centers) by 4.0 to 12.1 m in length. All studies were maintained by agronomic practices for optimal productivity. Seed used for testing was treated only with a commercial fungicide and no preventive treatments of soil or foliar applied insecticides were applied across the test area to plots not designated to receive such treatments.

Natural fall armyworm infestations or artificial fall armyworm infestations of 3 immature stages were used to evaluate efficacy of Cry1F maize hybrids. Artificial infestations occurred at intervals which corresponded to V4-V6, V4-V8, V5-V6, V6, V6-V7 and V7 stages of corn development (Ritchie et al. 1993). All plants within a single center row of each plot were infested at a particular interval and at each location. Plants were infested 1 or 2 times during each study. Fall armyworm eggs and larvae used in these studies were provided by Dow AgroSciences (Indianapolis, IN) and had originated from a collection in maize. Insects were shipped to test locations as egg masses laid on wax paper sheets or as 2nd instars reared on a meridic insect diet (Southland Multispecies Diet, Southland Products, Inc., Lake Village, AR) in 236-mL cups (1 egg mass per cup). Fall armyworm received as eggs were hatched and mixed with corncob grit and infested into plant whorls by the technique and a plastic dispensing device (bazooka) described by Davis & Oswalt (1979). Second instars were individually placed into the whorls of corn plants with a fine camel hair paint brush. Egg masses were cut from wax paper oviposition sheets and a single mass containing approximately 50 eggs was placed into an individual whorl. At 14 to 24 d after the establishment of either natural or artificial infestations, all plants were rated for leaf-feeding injury on a 0-9 scale (Davis et al. 1992), where 0 is no visible injury and 9 is whorl and furl leaves >90% destroyed.

### Laboratory and Field Studies on Cotton Producing Cry1F Bt Protein

Studies evaluating survival of fall armyworm on Cry1Ac:Cry1F cotton plants and non-Bt cotton varieties were conducted during 2004 to 2007 with fresh tissue laboratory bioassays or natural infestation field studies. At each test site, cultural practices including fertility, irrigation, and weed

TABLE 1. LOCATION AND METHODOLOGIES USED FOR ACROSS TRIAL SUMMARIZATIONS OF CRY1F EFFICACY, AS EXPRESSED IN MAIZE HYBRIDS, AGAINST FALL ARMYWORM IN THE UNITED STATES AND BRAZIL.

Year	Location	Cry1F (non-Bt isoline) Corn Hybrid Pairs Evaluated <sup>1</sup>	Methodology For Evaluating Efficacy <sup>2</sup>
2002	Fowler, IN Greenville, MS	2G768 (M2784) 2G768 (M2784)	Artificial infestation: neonate larvae (15/plant); applied at V7 maize growth stage Artificial infestation: eggs (15/plant) applied to V7 maize growth stage
2003	Fowler, IN	2G768 (M2784)	Artificial infestation: neonate larvae (30/plant) applied twice at V5-V6 and V6-V7 maize growth stage
2004	Fowler, IN	2G768 (M2784), 2A812 (2A775), 11084BMR (F717BMR)	Artificial infestation: neonate larvae (25/plant) applied twice 3-d apart at V5-V6 maize growth stage
2005	Fowler, IN  Huxley, IA	2G768 (M2784), 2A812 (2A775), 11084BMR (F717BMR)  2G768 (M2784), 2A812 (2A775), 11084BMR (F717BMR)	Artificial infestation: eggs (1 mass/plant, 50 eggs/mass) applied twice at V5-V6 and V6-V7 maize growth stage  Artificial infestation: Second instar larvae (10/plant) applied at V4-V8 maize growth stage
2006	Fowler, IN	2P788 (M2784)	Artificial infestation: second instar larvae (20/plant) at V6 maize growth stage
2007	Indianópolis, Minas Gerais, Brazil Rio Verde, Goiás, Brazil Jardinópolis, São Paulo, Brazil	2B710 HX (2B710) 2B710 HX (2B710) 2B710 HX (2B710)	Natural infestation to whorl stage maize Natural infestation to whorl stage maize Natural infestation to whorl stage maize

<sup>1</sup>Mycogen Cry1F and non-Bt maize hybrids.

<sup>2</sup>Maize growth stages described by Ritchie et al. (1993).

management, as recommended by state extension guidelines, were used to maintain experimental plots for optimum productivity. The entire test area was managed for non-lepidopteran insects pests, including thrips (Thysanoptera: Thripidae), aphids (Homoptera: Aphididae), stink bugs (Hemiptera: Pentatomidae), and plant bugs (Hemiptera: Miridae), by using insecticide chemistries with limited activity against lepidopteran insects. Insecticides used included aldicarb (Temik®150 g/kg, Bayer Crop Science, Research Triangle Park, NC), dicofen (Bidrin®480 g/L E, Amvac Chemical Corporation), and thiamethoxam (Centric®400 g/kg WG, Syngenta Crop Protection, Greensboro, NC). The data reported from the following studies is reflective of cotton managed without insecticides active against lepidopteran pests.

Plots were planted in a randomized block experimental design at each location. Treatments were replicated 4 times at each location, with exception of Wharton, TX and Pine Bluff, AR, which included 3 replications. At each location, single or multiple cotton varieties containing Cry1Ac:Cry1F were compared to a single gene or non-Bt cotton varieties. Cry1Ac:Cry1F cotton varieties included PHY 440 W, PHY 470 WR, PHY 475 WRF, PHY 485 WRF, and PHY 375 WRF (all from PhytoGen® Seed Company, LLC, Indianapolis, IN). Non-Bt cotton varieties included PSC 355, PHY 410 R, and PHY 315 RF. Stoneville 46971B, which produces Cry1Ac, was the single-gene Bt cotton variety evaluated. PhytoGen cotton varieties within the same series (i.e., 400s or 300s) are derived from the same parent rather than being genetic isolines. Plots sizes were 12.2 m long by 4 rows (91.4-101.6 cm centers) at all locations with the exception of Wharton, TX, where plots were 12 rows by 272.7 m. All studies were maintained by agronomic practices for optimal productivity.

In the natural infestation field studies, the center rows of each plot were sampled on a weekly basis beginning at the onset of anthesis and examined for the presence of fall armyworm larvae. At the Lonoke and Pine Bluff, AR locations during 2005, whole plant samples of 20 and 60 plants per plot, respectively, were conducted on each date. All plant structures (reproductive and vegetative components) were visually inspected for presence of fall armyworm larvae. At other locations during 2006 and 2007, 4 types of plant structures were sampled and included squares (flower bud), flowers, and bolls (fruit). Fruiting forms (40 squares, white flowers, and bolls per plot) were randomly selected and examined for presence of surviving fall armyworm larvae on each date. Larval count data for each plot was transformed to percent larval infestation based on the number of structures (i.e., 40) or whole cotton plants (i.e., 20 or 60) sampled as the denominator. For all nat-

ural infestation field trials, data reported are for the date of peak larval infestations in the non-Bt cotton variety.

In fresh tissue bioassays, fall armyworm larvae were infested on mature, fully expanded leaves. At the Stoneville, MS location, leaves were collected from all varieties when plants across the test plots had approximately 5 mainstem nodes above a sympodial branch with a flower on the first node (5 NAWF). Leaves collected were located 5 mainstem nodes below the terminal apex. First instars ( $F_2$  generation) were placed on an individual leaf inside a 9.2-cm diameter plastic Petri dish with a 9.0-cm diameter filter paper and covered to prevent escape (5 larvae per dish and 5 dishes per variety). Fall armyworm colonies used in each bioassay were derived from collections from one of 3 different plant hosts: cotton, bermudagrass, *Cynodon dactylon* (L.), and royal paulownia, *Paulownia tomentosa* (Thunb.). A subset of fall armyworm from these colonies were subjected to genetic analysis to determine their host-associated strain based on methods as described by Meagher & Gallo-Meagher (2003). At the Starkville, MS location, leaves were collected from cotton at the initiation of anthesis and were located 4 mainstem nodes below the terminal. A single 2.54-cm diameter disc was excised from each leaf and infested with a single, 1st instar fall armyworm (8 leaf discs per replication). Leaf discs were placed in individual cells (3.81 cm length  $\times$  4.44 cm width  $\times$  2.54 cm depth) of molded rearing trays (BIO-RT-32, C-D International, Pittman, NJ) and covered with perforated lids (Bio-CV4, C-D International, Pittman, NJ). An agar medium was added to each cell to maintain leaf turgor. Fall armyworm larvae used were established from crosses between wild males collected during 2004 and laboratory reared females that had previously been maintained in culture. Larval survival was determined at 4 to 5 d after infestation in each bioassay. The criterion for survival was the ability of a larva to make coordinated movement when prodded with a camel-hair brush.

#### Statistical Analysis

The 0-9 scale rating data obtained from the field studies in maize were analyzed within each country by multinomial, ordinal logistic regression techniques (Minitab 1998). Paired treatments were compared and *P*-values for the coefficient estimating change in the logit link function, odds of observing a 0 score for the first treatment in the pair relative to second treatment (odds ratio), and 95% confidence intervals were calculated. In addition, individual plant damage ratings within each replication were averaged and rounded to the nearest whole number. The frequency of occurrence of each of the 0-9 damage

rating values was tabulated across the trial sites and used to compare among the management strategies for fall armyworm.

For laboratory and field studies in cotton, percent larval survival and percent larval infestation data were subjected to Markov chain Monte Carlo (MCMC) simulations and similar to that of Mila & Michailides (2006). Analysis of variance techniques were not utilized because data sets were characterized by non-normal distributions, heterogeneity, and small number of observations. BRugs software (Bayesian inference with Gibbs sampling) was used to build 95% credible intervals which were used to compare treatments (R Development Core Team 2005; Thomas et al. 2006). Credible intervals provide the probability that a mean is contained within the calculated interval (Box-Steffensmeier et al. 2008). Treatment means were considered significantly different if 95% credible intervals did not overlap (Carlin & Lewis 2000).

## RESULTS AND DISCUSSION

### Efficacy of Corn Hybrid Producing Cry1F

Four Cry1F and 3 non-Bt maize hybrids were compared in 7 U.S. field trials for leaf-feeding injury by fall armyworm. Mean ( $\pm$  SEM) injury ratings for Cry1F-Bt maize hybrids including 11083BMR, 2A812, 2G768, and 2P788 were  $1.4 \pm 0.5$ ,  $1.5 \pm 0.8$ ,  $0.9 \pm 0.7$ , and  $1.7 \pm 1.7$ , respectively. Mean ( $\pm$  SEM) injury ratings for non-Bt maize hybrids including 2A775, F717BMR, and M2784 were  $7.4 \pm 0.5$ ,  $8.1 \pm 1.0$ , and  $7.9 \pm 1.1$ , respectively. There was no significant difference in damage among non-Bt corn hybrids (treatment pair, *P*-value, odds ratio, 95% confidence limits: M2784 and F717BMR, 0.693, 1.32, 0.33-5.31; 2A775 and F717BMR, 0.151, 3.77, 0.62-23.06; 2A775 and M2784, 0.137, 2.85, 0.72-11.33; F717BMR and M2784, 0.693, 0.76, 0.19-3.04). In addition, there was no significant difference in damage among Cry1F-Bt corn hybrids (treatment pair, *P*-value, odds ratio, 95% confidence limits: 2G768 and F11084BMR, 0.174, 2.96, 0.62-14.01; 2A812 and F11084BMR, 0.868, 0.86, 0.13-5.42; 2P788 and F11084BMR, 0.937, 1.07, 0.18-6.24; 2A812 and 2G768, 0.120, 0.29, 0.06-1.38; 2P788 and 2G768, 0.170, 0.36, 0.09-1.54; F11084BMR and 2G768, 0.174, 0.34, 0.07-1.61; 2P788 and 2A812, 0.800, 1.26, 0.22-7.26; F11084BMR and 2A812, 0.868, 1.17, 0.18-7.41; 2G768 and 2A812, 0.120, 3.46, 0.72-16.56). Therefore, injury ratings for all non-Bt and all Cry1F-Bt maize hybrids were combined for analysis.

Mean leaf-feeding injury for Cry1F-Bt maize hybrids was 1.3 as compared to 7.9 for the non-Bt corn hybrids (Fig. 1). Injury was significantly less for Cry1F-Bt maize hybrids as compared to

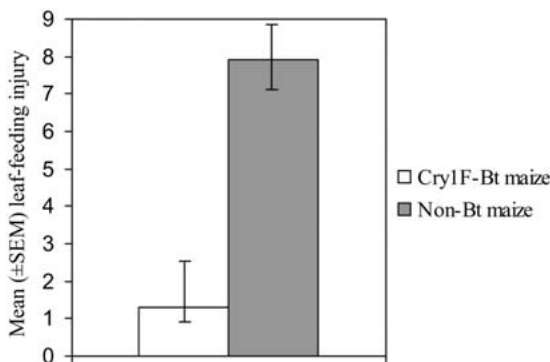


Fig. 1. Leaf-feeding injury (0-9 scale, Davis et al. 1992) by fall armyworm on a Bt and non-Bt maize hybrid, 2002-2006, U.S.

non-Bt maize hybrids (*P*-value, odds ratio, 95% confidence intervals:  $<0.001$ , 0.00, 0.00-0.00). Therefore, it is highly unlikely that the lower damage ratings would be observed on non-Bt maize hybrids relative to the Cry1F-Bt maize hybrids. These results were consistent with field trials reported in the southern U.S. by Siebert et al. (2008) in which Cry1F-Bt maize hybrids provided significant protection and improved plant height when exposed to high levels of feeding pressure from fall armyworm. In addition, Waquil et al. (2002) demonstrated that a maize hybrid containing Cry1F provided better control of fall armyworm feeding than hybrids producing either Cry1Ab or native host plant resistance factors.

The use of multinomial techniques for the Brazil data was not possible (model convergence not obtained) and was likely due to the small data set. Leaf feeding injury for the Cry1F-Bt and non-Bt maize hybrids was  $1.3 \pm 0.4$  and  $4.0 \pm 1.9$ , respectively, similar to the trends observed in the U.S. data. Level of injury for non-Bt maize managed with foliar insecticides was  $4.1 \pm 1.5$ . The range of damage values assigned to Cry1F-Bt maize hybrids, a foliar insecticide program, and non-Bt maize hybrids were 1-2, 2-7, and 2-7, respectively, (Fig. 2). Based on this qualitative analysis, managing fall armyworm with foliar insecticides on non-Bt corn was similar to damage levels observed on non-Bt maize hybrids. Furthermore, damage values for these non-Bt strategies were generally greater as compared to a management strategy with Cry1F-Bt maize hybrids. A multinomial logistic regression analysis of the combined U.S. and Brazil Cry1F-Bt and non-Bt data produced results similar to that of the U.S. alone (*P*-value, odds ratio:  $<0.001$ , 0.00). These results reinforce the improbability of observing a damage rating lower for a non-Bt maize hybrid relative to a Cry1F-Bt maize hybrid across geographies.

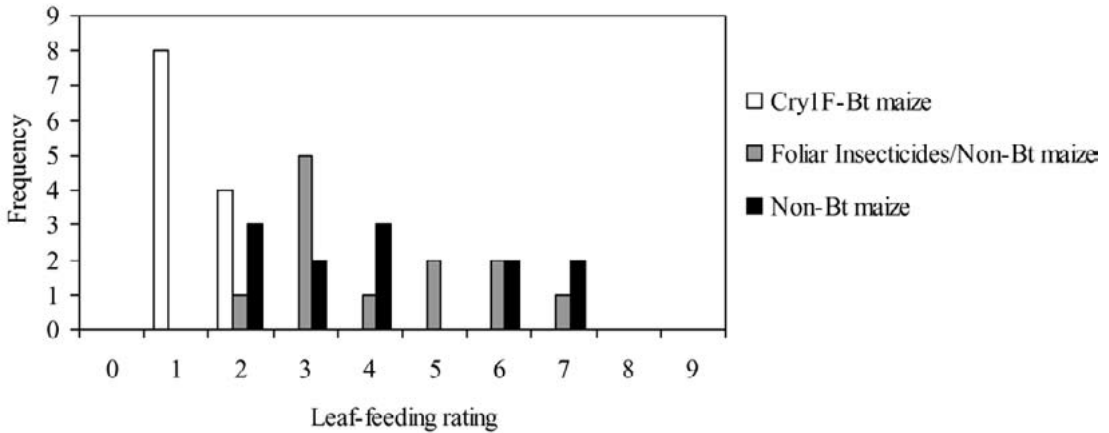


Fig 2. Comparison of transgenic and non-transgenic management tactics for control of fall armyworm on maize, 2005, Brazil.

Maize hybrids containing Cry1F have provided effective control of fall armyworm in Argentina field trials. In Los Altos, Catamarca grain yields were significantly greater (17%,  $P < 0.05$ ) for a corn hybrid containing Cry1F as compared to a non-Bt corn hybrid (Dow AgroSciences, LLC, unpublished data). The results reported here demonstrate that Cry1F maize hybrids can serve as an effective management option for fall armyworm in South America. In addition, management of fall armyworm with Cry1F may be a better management option as compared to using foliar insecticides on non-Bt maize.

Efficacy of Cotton Varieties Producing Cry1F

Survival of fall armyworm larvae did not exceed 4.0% in laboratory infestations on mature leaves of PHY 440 W, which contains Cry1Ac:Cry1F (Table 2). Regardless of the source (plant host) of laboratory-reared fall armyworm, larval survival on PHY 440 W was significantly less than on PSC355 cotton ( $P < 0.05$ ). Survival of fall armyworm larvae on Stoneville 4691B, which contains Cry1Ac, and Stoneville 474 (non-Bt) was not significantly different than PSC 355 (non-Bt) cotton ( $P > 0.05$ ). Additionally, fall armyworm

TABLE 2. SURVIVAL OF FALL ARMYWORM (FIRST INSTAR) FROM VARIOUS LABORATORY COLONIES EXPOSED TO NON-BT AND BT COTTON TISSUE IN A FRESH TISSUE BIOASSAY OF MATURE LEAVES

Variety	Percent Survival $\pm$ SEM (95% Credible Interval)			
	Stoneville, MS: Source of Laboratory Reared Fall Armyworm <sup>1</sup>			Starkville, MS <sup>2</sup>
	Cotton <sup>3</sup>	Bermudagrass <sup>4</sup>	Royal Paulownia <sup>4</sup>	
PSC 355	96.0 $\pm$ 8.9 (68.8 - 95.5)	88.0 $\pm$ 17.9 (59.5 - 97.8)	96.0 $\pm$ 8.9 (68.7 - 99.3)	81.5 $\pm$ 16.1 (69.0 - 99.3)
Stoneville 474	72.0 $\pm$ 17.9 (47.7 - 92.6)	72.0 $\pm$ 26.8 (47.3 - 94.2)	84.0 $\pm$ 21.9 (55.4 - 97.1)	—
Stoneville 4691B <sup>5</sup>	64.0 $\pm$ 26.1 (41.7 - 90.5)	60.0 $\pm$ 14.1 (43.7 - 73.6)	84.0 $\pm$ 21.9 (56.2 - 97.2)	—
PhytoGen 440 W <sup>6</sup>	4.0 $\pm$ 8.9 (0.7 - 30.7)	4.0 $\pm$ 8.9 (0.7 - 31.4)	4.0 $\pm$ 8.9 (0.7 - 31.1)	0.0 $\pm$ 0.0

Mean survival within columns are significantly different if 95% credible intervals do not overlap ( $\alpha = 0.05$ , MCMC simulations).

<sup>1</sup>Mature leaves located five mainstem nodes below the terminal. Evaluation at 4 d after infestation.

<sup>2</sup>Mature leaves located four mainstem nodes below the terminal. Evaluation at 5 d after infestation.

<sup>3</sup>Corn-associated strain as determined by genetic analysis.

<sup>4</sup>Rice-associated strain as determined by genetic analysis.

<sup>5</sup>Cotton containing Cry1Ac (MON 531) Bt protein.

<sup>6</sup>Cotton containing Cry1Ac (DAS-21023-5) and Cry1F (DAS-24236-5) Bt proteins.

survival was significantly less on PHY 440 W as compared to Stoneville 4691B.

At Stoneville, MS, genetic analysis of fall armyworm collections indicated the larvae originating from bermudagrass and royal paulownia to be of the rice-associated strain and the colony originating from cotton to be of the corn-associated strain. Pashley (1986) described these fall armyworm host strains, which exhibit polymorphisms at 5 allozyme loci. Identification of fall armyworm host strains is important because differences in susceptibility to insecticides and Cry1Ac Bt toxin have been demonstrated (Pashley et al. 1987; Adamczyk et al. 1997). Adamczyk et al. (1997) demonstrated that larvae collected from bermudagrass and browntop millet, *Brachiaria ramosa* (L.), were significantly more sensitive to Cry1Ac Bt cotton as compared to larvae collected from maize. In contrast, results from our studies indicate that corn-associated and rice-associated host strains of fall armyworm are equally sensitive to cotton producing Cry1Ac and Cry1F combined, while little control of either strain was evident in the Cry1Ac-only cotton variety.

In field studies, varieties containing Cry1Ac:Cry1F significantly reduced ( $P < 0.05$ ) larval infestations in whole plants as compared to a non-Bt cotton variety, PHY 410 R, across 2 locations (Table 3). There were 6.3, 16.0, and 4.5-fold reductions in larvae infestations for PHY 440 W, PHY 470 WR, and PHY 475 WRF, respectively, as compared to PHY 410 R, at Lonoke, AR. Similarly at Pine Bluff, AR, there was a 10.6-fold reduction for PHY 470 WR as compared to PHY 410 R. In addition, at the Lonoke, AR location there was no significant difference in larval infestations among varieties containing Cry1Ac:Cry1F.

Larval infestations were significantly ( $P < 0.05$ ) reduced in samples of squares, flowers, and bolls for varieties containing Cry1Ac:Cry1F in Wharton, TX, during 2006 as compared to a non-Bt cotton variety (Table 4). Across structures, percent

larval infestation of PHY 425 RF (non-Bt) and PHY 485 WRF (Cry1Ac:Cry1F) ranged from 1.7 to 13.3% and 0.0 to 0.8%, respectively. Larval infestations were significantly ( $P < 0.05$ ) reduced in samples of bolls for PHY 485 WRF and PHY 375 WRF in Lonoke, AR during 2007 as compared to their respective non-Bt cotton varieties (Table 4). Significant differences were not detected for larval infestations in flowers at the Lonoke, AR location for either the PHY 400 or 300 varietal series. Across structures and varieties, percent larval infestation of non-Bt varieties and Cry1Ac:Cry1F varieties ranged from 2.5 to 4.4% and 0.0 to 0.6%, respectively.

Previous studies by Adamczyk & Gore (2004b) have established that Cry1F Bt protein, rather than Cry1Ac, in a Cry1Ac:Cry1F variety provides control of fall armyworm and that synergism between the two proteins was not apparent. In addition, commercial cotton varieties containing Cry1Ac (event MON 531) have not provided commercially acceptable control of fall armyworm (Stewart et al. 2001). Similar results were observed in our laboratory bioassay in that survival was similar between Stoneville 4691B (Cry1Ac) and PSC 355 (non-Bt) cotton across three colonies of fall armyworm. Therefore our results support the conclusions of Adamczyk & Gore (2004b), that control provided by varieties containing Cry1Ac and Cry1F is attributed to the presence of Cry1F Bt protein.

Fall armyworm infestations are initially established on mature leaves and these studies have demonstrated high levels of mortality (96%) with a variety containing Cry1F. Coincidentally, expression of Cry1F protein is greatest in mature leaves as compared to other structures including terminal leaves, squares, flowers, and bolls and protein levels in mature leaves increases with age (Dow AgroSciences, LLC, unpublished data). Assuming there is a positive relationship between protein expression and control of target insects in

TABLE 3. COMPARISON OF COTTON VARIETIES CONTAINING CRY1AC:CRY1F AND A NON-BT VARIETY FOR CONTROL OF FALL ARMYWORM IN NATURAL INFESTATION FIELD STUDIES, 2005.

Variety	Percent Larval Infestation <sup>1</sup> ± SEM (95% Credible Interval)	
	Lonoke, AR	Pine Bluff, AR
PHY 440 W <sup>2</sup>	6.3 ± 4.8 (2.2 - 12.6)	—
PHY 470 WR	2.5 ± 5.0 (0.3 - 7.0)	5.6 ± 6.7 (0.2 - 3.2)
PHY 475 WRF	8.8 ± 8.5 (3.7 - 16.0)	—
PHY 410 R	40.0 ± 10.8 (29.7 - 50.8)	59.4 ± 39.3 (59.0 - 72.9)

Mean larval infestations within columns are significantly different if 95% credible intervals do not overlap ( $\alpha = 0.05$ , MCMC simulations).

<sup>1</sup>Peak date of fall armyworm infestations based on percent larval infestation in PHY 410 R non-Bt treatment.

<sup>2</sup>Cotton varieties containing Cry1Ac (DAS-21023-5) and Cry1F (DAS-24236-5)Bt proteins are denoted by 'W'.



TABLE 4. COMPARISON OF A CRY1Ac:CRY1F COTTON VARIETY AND A RELATED NON-BT COTTON FOR CONTROL OF FALL ARMYWORM IN NATURAL INFESTATION STUDIES.

Location/Year	Structure	Percent Larval Infestations $\pm$ SEM (95% Credible Interval) <sup>1</sup>	
		PHY 485 WRF <sup>2</sup>	PHY 425 RF
Wharton, TX 2006	Flower	0.8 $\pm$ 1.4 (0.03 – 3.2)	13.3 $\pm$ 8.0 (7.9 – 20.0)
	Square	0.0 $\pm$ 0.0	3.3 $\pm$ 1.4 (1.0 – 7.3)
	Boll	0.0 $\pm$ 0.0	1.7 $\pm$ 2.9 (0.2 – 4.7)
Lonoke, AR 2007		PHY 485 WRF	PHY 425 RF
	Flower	0.6 $\pm$ 1.3 (0.02 – 2.5)	2.5 $\pm$ 3.5 (0.7 – 5.5)
	Boll	0.0 $\pm$ 0.0	4.4 $\pm$ 3.1 (1.8 – 8.2)
		PHY 375 WRF	PHY 315 RF
	Flower	0.6 $\pm$ 1.3 (0.02 – 2.5)	2.5 $\pm$ 2.0 (0.7 – 5.5)
	Boll	0.0 $\pm$ 0.0	3.8 $\pm$ 1.4 (1.4 – 7.3)

<sup>1</sup>Means larval infestations within rows are significantly different if 95% credible intervals do not overlap ( $\alpha = 0.05$ , MCMC simulations).

<sup>2</sup>Peak date of fall armyworm infestations based on percent larval infestation in PHY 425 RF and PHY 315 RF non-Bt cotton varieties.

<sup>3</sup>Cotton varieties containing Cry1Ac (DAS-21Ø23-5) and Cry1F (DAS-24236-5) Bt proteins are denoted by 'W'.

the field, it is plausible that increasing levels of Cry1F protein in leaves as plants mature could support these findings.

Larvae that survive on mature leaf tissue in the field will presumably move to cotton squares, flowers, and bolls. In our field studies, cotton varieties containing Cry1Ac:CRY1F had significantly reduced fall armyworm larval densities in these structures as compared to that of non-Bt cotton lines. In addition, field efficacy of Cry1Ac:CRY1F cotton was confirmed for multiple varieties (PHY 440 W, PHY 470 WR, PHY 475 WRF, PHY 485 WRF, PHY 375 WRF). It has been documented that where protein expression for 2 Cry1Ac cotton varieties differed the survival of bollworm also differed (Adamczyk & Gore 2004a). The results from our studies suggest deviations in efficacy among commercial varieties containing combined Cry1Ac (event DAS-21Ø23-5) and Cry1F (event DAS-24236-5) for fall armyworm control should not be anticipated based on a field study in Lonoke, AR which compared PHY 440 W, PHY 470 WR, and PHY 475 WRF.

Results from our field and laboratory studies evaluating insecticidal Cry1F Bt protein, as expressed in maize hybrids and cotton varieties, indicate economical levels of efficacy against fall armyworm. These results were validated in field trials conducted in numerous geographies ranging from the midwestern to the southern U.S. and in Brazil. In addition, Cry1F was confirmed to be efficacious against a broad range of native fall armyworm infestations, including two host-associated strains. In cotton, efficacy was also demonstrated for several plant structures commonly injured by fall armyworm. These results collectively demonstrate that maize and cotton varieties pro-

ducing Cry1F can be an important component of an overall management program for fall armyworm across a broad range of geographies.

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