Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) on transgenic field corn expressing *Bacillus thuringiensis* Cry proteins

Jarrod T. Hardkea,*, B. Rogers Leonardb, Fangneng Huanga, R.E. Jacksonc

*a Department of Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA
b Macon Ridge Research Station, Louisiana State University Agricultural Center, Winnsboro, LA 71295, USA
c USDA-ARS, Southern Insect Management Research Unit, Stoneville, MS 38776, USA

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**Abstract**

Field corn, *Zea mays* L., plants expressing Cry1Ab and Cry1F insecticidal crystal (Cry) proteins of *Bacillus thuringiensis* (Bt) Berliner are planted on considerable acreage across the Southern region of the United States. The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is an economically important pest during the mid-to-late season on non-Bt and some commercial Bt corn hybrids. The objective of this study was to quantify foliar injury and survivorship of fall armyworm on transgenic corn lines expressing Cry1Ab or Cry1F Bt proteins. Corn lines/hybrids expressing Cry1Ab, Cry1F, and a conventional non-Bt cultivar were evaluated against artificial infestations of fall armyworm in field trials. Larvae (second instars) of fall armyworm were placed on corn plants (V8-V10 stages). Leaf injury ratings were recorded 14 d after infestation. Hybrids expressing Cry1F had significantly lower feeding injury ratings than non-Bt corn plants. Development and survivorship of fall armyworm on Bt corn lines/hybrids were also evaluated in no-choice laboratory assays by offering freshly harvested corn leaf tissue to third instars. Transgenic corn hybrids expressing Cry1Ab or Cry1F significantly reduced growth, development, and survivorship of fall armyworm compared to those offered non-Bt corn tissue. However, 25–76% of third instars offered Bt corn leaf tissues successfully pupated and emerged as adults. These results suggest Cry1Ab has limited effects on fall armyworm; whereas Cry1F demonstrated significant reductions in foliar injury and lower survivorship compared to that on non-Bt corn tissues. Although fall armyworm is not considered a primary target for insect resistance management by the U.S. Environmental Protection Agency, these levels of survivorship could impact selection pressures across the farmscape, especially when considering that transgenic Bt cotton cultivars express similar Cry (Cry1Ac or Cry1F) proteins.

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1. Introduction

The first generation transgenic field corn, *Zea mays* L., hybrids expressing *Bacillus thuringiensis* (Bt) Berliner insecticidal crystal (Cry) proteins were introduced in the Southern United States during 1998 (Buntin et al., 2000, 2004; Castro et al., 2004). Since then, adoption of Bt corn has greatly increased because of high efficacy against target pests and ease-of-use for producers. The acreage of Bt corn cultivars has increased across this region and nearly saturated the level (50% of the total corn acreage) allowed by the U.S. Environmental Protection Agency.

Transgenic Bt corn hybrids were initially developed to reduce injury from corn stalk-boring pests such as the European corn borer, *Ostrinia nubilalis* (Hübner), and southwestern corn borer, *Diatraea grandiosella* (Dyar) (Abel et al., 2000; Buntin et al., 2004; Castro et al., 2004). Although the primary targets were corn stalk-borers, Cry1Ab corn also suppressed foliar damage from corn earworm, *Helicoverpa zea* (Boddie), and fall armyworm, *Spodoptera frugiperda* (J.E. Smith), infestations. These two species can be important yield- and quality-limiting pests in Southern U.S. corn fields (Buntin et al., 2004; Chilcutt et al., 2007). The first Bt corn hybrids expressed a single Bt protein, Cry1Ab, under the umbrella of YieldGard® (Monsanto Co., St. Louis, MO) technology and have been the most common Bt corn hybrids across the Southern region (Buntin et al., 2004; Huang et al., 2006).

The success of YieldGard prompted rapid development of other Bt technologies in field corn to further improve corn pest management strategies. In 2003, corn hybrids expressing Cry1F from Bt var. *aizawai* Berliner became commercially available.
The fall armyworm has historically been one of the most common pests of field corn in the Southern U.S. (Pitre and Hogg, 1983; Buntin, 1986; Buntin et al., 2004). This pest has a wide host range of more than 80 plant species and does not overwinter in most of the corn-production regions of the U.S. Each year, populations migrate from areas including south Florida, Caribbean islands, south Texas, Mexico, or Central America (Sparks, 1979; Adamczyk et al., 1997; Buntin, 1986) with the adults ovipositing on seasonal hosts during a northerly migration. Conventional chemical control strategies are inconsistent and often provide unsatisfactory control of the fall armyworm in field corn. Almost immediately after larval hatching, neonates move into the whorl region of corn plants where they are protected from foliar insecticides (Harrison, 1986; Castro, 2002; Bokonon-Ganta et al., 2003; Siebert et al., 2008a). Those insecticides which are generally efficacious against other pests, such as the corn earworm, typically provide limited control of fall armyworm (Young, 1979; Guillebeau and All, 1990). In addition, regional populations of fall armyworm have developed resistance to several classes of insecticides including carbamates, organophosphates, and pyrethroids (Adamczyk et al., 1999).

Fall armyworm infestations are frequently reported across the Southern region of the U.S. in conventional non-Bt and Bt (Cry1Ab) varieties, especially when fields are planted after the optimum seeding dates. Previous studies have evaluated the field efficacy of transgenic Bt corn against fall armyworm (Buntin et al., 2000, 2004; Buntin, 2008; Siebert et al., 2008a). However, most of the past work has focused only on foliar damage. Information on fall armyworm survival on Bt corn plants is very limited. A recent study (Siebert et al., 2008b) showed that larval survivorship (neonate-pupal stages) of fall armyworm was low on Cry1F Bt corn leaf tissue compared to that on conventional non-Bt tissue. The agrochemical industries consistently need additional data to support the use of Bt corn in controlling secondary lepidopteran pests such as fall armyworm, and knowledge of late instar survivorship on Bt plants can be important in the design of IRM strategies (Walker et al., 2000).

The objective of this study was to evaluate the effectiveness of the two most commonly used Bt corn technologies (e.g. YieldGard corn borer and Herculex I) against fall armyworm in the Southern region of the U.S. In this study, field trials were conducted to evaluate plant injury of Bt and non-Bt corn from artificial infestations of fall armyworm. Laboratory studies evaluated survivorship of late-instar on these two Bt corn technologies. The results generated from this study should provide useful information in developing new Bt corn technologies for managing fall armyworm.

2. Materials and methods

2.1. Fall armyworm colony establishment and maintenance

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

For each test, a cohort of 50 healthy pupae were removed from the colony, placed into plastic buckets (3.79 l), and covered with cheesecloth. Upon adult eclosion, adults were fed a 10% sucrose: water solution and allowed to mate. Eggs on cheesecloth sheets were allowed to hatch and larvae were reared on meridic diet until reaching the size needed for experiments.

2.2. Field trials

Field studies were conducted near Winnisiboro, Louisiana in Franklin Parish (32° 8’ 8” N, 91° 41’ 23” W) during 2007 and 2008. The experimental design used in 2007 was a randomized complete block with four replications. Plot size was 4 rows (centered on 1 m) and 9.14 m long. The corn lines evaluated during 2007 included Dekalb DKC 69-43 RR2 [Roundup Ready 2] (non-Bt) (DeKalb Seeds, Monsanto Corp., St. Louis, MO); Dekalb DKC 69-71 YGCB [YieldGard Corn Borer]/RR2 (Cry1Ab), and a Dow AgroSciences near isolate 27787 (Cry1F), and were planted on April 23, 2007. Corn lines tested during 2008 included Dekalb DKC 63-45 RR2 (non-Bt), Pioneer Brand 32B29 YGCB/RR2 (Cry1Ab) (Pioneer Hi-Bred International, Des Moines, IA), and Pioneer Brand 31C71 HX [Hercules]/RR2/LL [Liberty Link]/Cry1F. Three blocks of each variety were planted on each of four sequential planting dates. Each date of planting was used as a single replication.

For each in-field infestation event, 10 second instars (3–6 days old) were infested on the first fully-exposed leaf sheath from the top of a single V8-V10 stage plant. Plant stages selected for the insect infestations were based on descriptions by Morrill and Greene (1973), which indicated that the highest fall armyworm infestations occurred on early- to late-whorl stage corn plants. Five- to ten-consecutive plants in rows two or three were infested within each plot. In the 2007 trials, two infestation events were made on different plants within each plot. For the 2008 tests, there were three infestation events, each corresponding to a different planting date. At 14 d after infestation (DAI), all infested plants were visually inspected to record leaf damage ratings using a modified version of the injury scale recommended by Guthrie et al. (1960). The original scale bases injury ratings on leaves throughout the entire plant profile. The modified scale only evaluated new leaves above the point of larval infestation. Foliar damage ratings were summarized for all plants within each plot and used to calculate a single mean. These data were analyzed using a one-way analysis of variance with PROC MIXED (SAS Institute, 2004). For 2008 trials, planting date (or insect infestation event) was considered as a block analysis. Means were estimated using the LSMEANS statement and compared according to Dunnett’s test of difference from control (SAS Institute, 2004). The results for Cry1Ab hybrids were not compared to that of Cry1F hybrids to maintain compliance with contractual requirements from the participating agrochemical and seed industries. Results for each Bt hybrid were independently compared to the non-Bt control. Different corn hybrids were used during each growing season and therefore data were not combined across years.

2.3. No-choice leaf tissue bioassays

During 2007–2009, third instars (30–45 mg/larva) were offered fresh corn leaf tissue removed directly from field plots. During 2007 and 2008, corn hybrids and experimental designs were the same as those previously described for the field trials described above. For the 2009 bioassays, corn hybrids included Pioneer 31P40 RR2 (non-Bt), Dekalb DKC 67-03 YGCB/RR2 (Cry1Ab), and Pioneer 31P42 HX/RR2/LL (Cry1F). There were three sequential planting dates with
Table 1
Fall armyworm survivorship and development (mean, SEM) on non-Bt and Bt corn hybrids in no-choice leaf tissue bioassays, Winnsboro, LA, 2007.

<table>
<thead>
<tr>
<th>Life History Trait</th>
<th>Non-Bt</th>
<th>Cry1Ab</th>
<th>Cry1F</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval survivorship (%)</td>
<td>66.0</td>
<td>62.0</td>
<td>36.7</td>
<td>4.11</td>
<td>2, 6</td>
<td>0.0751</td>
</tr>
<tr>
<td>Larval duration (d)</td>
<td>12.1</td>
<td>14.9</td>
<td>16.5</td>
<td>33.19</td>
<td>2, 6</td>
<td>0.0006</td>
</tr>
<tr>
<td>Pupal duration (d)</td>
<td>6.6</td>
<td>9.0</td>
<td>8.2</td>
<td>0.89</td>
<td>2, 6</td>
<td>0.4581</td>
</tr>
<tr>
<td>Adult eclosion (%)</td>
<td>56.7</td>
<td>43.3</td>
<td>25.6</td>
<td>3.79</td>
<td>2, 6</td>
<td>0.0862</td>
</tr>
</tbody>
</table>

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

Table 2
Fall armyworm survivorship and development (mean, SEM) on non-Bt and Bt corn hybrids in no-choice leaf tissue bioassays, Winnsboro, LA, 2008.

<table>
<thead>
<tr>
<th>Life History Trait</th>
<th>Non-Bt</th>
<th>Cry1Ab</th>
<th>Cry1F</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval survivorship (%)</td>
<td>88.0</td>
<td>88.9</td>
<td>31.3</td>
<td>92.92</td>
<td>2, 6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Larval duration (d)</td>
<td>9.5</td>
<td>10.2</td>
<td>12.4</td>
<td>14.30</td>
<td>2, 6</td>
<td>0.0052</td>
</tr>
<tr>
<td>Pupal weight (mg)</td>
<td>171.2</td>
<td>163.4</td>
<td>157.5</td>
<td>8.05</td>
<td>2, 6</td>
<td>0.0200</td>
</tr>
<tr>
<td>Pupal duration (d)</td>
<td>9.8</td>
<td>8.7</td>
<td>9.3</td>
<td>2.02</td>
<td>2, 6</td>
<td>0.2129</td>
</tr>
<tr>
<td>Adult eclosion (%)</td>
<td>78.7</td>
<td>75.6</td>
<td>24.7</td>
<td>41.52</td>
<td>2, 6</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Means within rows followed by an asterisk (*) are significantly different from the non-Bt (P ≤ 0.05, Dunnett’s).
Cry1F corn leaf tissue (present study) was considerably greater than that of neonates on Bt corn plants. The observation, the Cry1F protein significantly decreased plant injury and inhibited fall armyworm development. In the event of extreme pressure from native fall armyworm infestations, corn lines expressing Cry1F still may not provide sufficient control of this insect, especially if protein expression in all tissue is not sufficiently high to cause mortality. In addition, fall armyworm prefers to feed on young leaves in the whorl (Morrill and Greene, 1973; Harrison, 1984). Studies have shown that Cry1Ab expression was lowest in the younger leaves of the uppermost area of the plant (Abel and Adamczyk, 2004). If this same characteristic is true of the Cry1F-expressing corn hybrids used in this test, this may explain the ability of fall armyworm to tolerate exposure to either Bt protein and for some larvae to successfully complete development.

Resistance development in target insect pests is a major concern for the sustainable use of Bt crop technologies (United States Environmental Protection Agency, 2001). The initial reports of field-derived resistance that resulted in control failures with Bt corn crops included a fall armyworm population in Puerto Rico on Cry1F-expressing corn during 2006 (Matten et al., 2008; Tabashnik et al., 2009; Storer et al., 2010). Past and current studies clearly suggest that neither the YieldGard (Cry1Ab) nor Herculex I (Cry1F) technologies express a “high dose” against fall armyworm as defined by an EPA FIFRA Scientific Advisory Panel (1998) working on Bt resistance management (United States Environmental Protection Agency, 2001). Many factors might have contributed to Cry1F resistance in the Puerto Rico fall armyworm population. The lack of a high dose expressed in Herculex I hybrids for fall armyworm could be a major reason for resistance development to Cry1F. The island geography of Puerto Rico, unique climatic conditions, and limited availability of alternate hosts favorable for fall armyworm could serve to intensify fall armyworm infestations on crops. In addition, heavy reliance on Bt foliar sprays could have predisposed this population to Bt resistance development (Storer et al., 2010). Ultimately, high numbers of healthy individuals intensively selected with Bt could have changed the susceptibility of this population.

Presently, a seed mixture (Bt and non-Bt corn, so called “refuge-in-a-bag”) is currently widely discussed as an alternative refuge strategy for Bt resistance management. The high survivorship of fall armyworm on Bt corn leaf tissue observed in the current study suggests that late instars could migrate from non-Bt plants in the seed mix and survive on transgenic Bt plants and cause plant injury. The presence of alternate weedy hosts or corn plants not expressing Bt proteins could support interplant migration of larvae and an increase in injury to Bt-expressing plants (Storer et al., 2010). This plant-to-plant movement could create another level of selection pressure for the development of resistance in fall armyworm populations that has not been previously considered. With the recent introduction of pyramided Bt proteins in field corn, non-Bt corn refuge requirements have been reduced (United States Environmental Protection Agency, 2010). In cotton, plants expressing only Cry1Ac were phased out during 2010. Producers planting cotton cultivars with pyramided Bt proteins are not required to have any associated non-Bt cotton refuge. Across the Southern region,

### Table 3

<table>
<thead>
<tr>
<th>Life History Trait</th>
<th>Non-Bt</th>
<th>Cry1Ab</th>
<th>Cry1F</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval survivorship (%)</td>
<td>91.7</td>
<td>82.6</td>
<td>62</td>
<td>64.4</td>
<td>2.9*</td>
<td>0.0067</td>
</tr>
<tr>
<td>Larval duration (d)</td>
<td>10.1</td>
<td>10.9</td>
<td>0.5*</td>
<td>11.2</td>
<td>0.4*</td>
<td>0.0078</td>
</tr>
<tr>
<td>Pupal weight (mg)</td>
<td>170.5</td>
<td>139.2</td>
<td>11.0*</td>
<td>172.0</td>
<td>5.8</td>
<td>0.0114</td>
</tr>
<tr>
<td>Pupal duration (d)</td>
<td>9.4</td>
<td>10.2</td>
<td>0.2</td>
<td>10.2</td>
<td>0.0</td>
<td>0.2216</td>
</tr>
<tr>
<td>Adult eclosion (%)</td>
<td>85.0</td>
<td>71.5</td>
<td>8.1</td>
<td>55.6</td>
<td>5.9*</td>
<td>0.0237</td>
</tr>
</tbody>
</table>

Means within rows followed by an asterisk (*) are significantly different from the non-Bt (P = 0.05, Dunnett’s).
field corn and cotton are two of the primary crop hosts for fall armyworm, and the lack of an associated non-Bt crop refuge with either crop may greatly influence Bt selection pressure in this species.

In general, our results suggest that caution must be taken in wide use of these Bt corn technologies as individual and independent products for controlling fall armyworm in the Southern region, especially in areas where this species can successfully overwinter. Pyramid Bt proteins will likely result in a broader spectrum of insect pest control and improve fall armyworm management.

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References


Abel, C.A., Adamczyk, Jr., J.J., 2004. Relative concentration of Cry1A in maize leaves and cotton bolls with diverse chlorophyll content and corresponding larval development of fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer (Lepidoptera: Crambidae) on maize whorl leaf profiles. J. Econ. Entomol. 97, 1737–1744.


