Crop Protection 30 (2011) 168–172

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

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

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ARTICLE INFO

Article history:
Received 23 March 2010
Received in revised form
17 October 2010
Accepted 18 October 2010

Keywords:
Spodoptera frugiperda
Zea mays
Larval-pupal survivorship
Cry1F
Cry1Ab

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.

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.

☆ This paper reports results only. Mention of a proprietary product name does not constitute an endorsement for its use by Louisiana State University Agricultural Center or United States Department of Agriculture Agricultural Research Service.

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0261-2194/$ – see front matter
doi:10.1016/j.cropro.2010.10.005

Hybrids expressing this trait have been trademarked as Herculex I™ Insect Protection (Dow AgroSciences, Indianapolis, IN & Pioneer Hi-Bred International, Des Moines, IA). Similar to the YieldGard technology, Herculex has been reported to be highly effective against corn stalk-borers and provides limited suppression of corn earworm (Baldwin et al., 2009; Siebert et al., 2008a). In addition, Herculex lists several other lepidopteran pests as targets, including the fall armyworm (Baldwin et al., 2009).

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 insecticide sprays (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-instars 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 Winnsboro, LA during 2005 and was supplemented with collections from field corn in the same area during 2006 and 2008. The colony was validated as the corn strain of fall armyworm using mitochondrial markers (unpublished communication, R. Nagoshi, USDA-ARS, Gainesville, FL). The colony has been maintained in the laboratory on meridic diet (Stonefly 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 Winnsboro, 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 Comp., St. Louis, MO), Dekalb DKC 69-71 YGCB [YieldGard Corn Borer]/RR2 (Cry1Ab), and a Dow AgroSciences near isoline 2T787 (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 31G71 HX [Herculex]/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