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Larval survival and plant injury of Cry1Ab-susceptible, -resistant, and -heterozygous genotypes of the sugarcane borer on transgenic corn containing single or pyramided Bt genes

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Abstract
Transgenic corn (*Zea mays* L.) products expressing multiple Bt proteins targeting a same group of insect pests have become commercially available in the U.S. The first commercialized pyramided Bt corn technologies for managing lepidopteran pests include Genuity® VT Triple Pro™ and Genuity® SmartStax™. In this study, larval survival and plant injury of Cry1Ab-susceptible (Cry1Ab-SS), -resistant (Cry1Ab-RR), and -heterozygous (Cry1Ab-RS) genotypes of the sugarcane borer, *Diatraea saccharalis* F., on five commercial corn hybrids were evaluated in a leaf tissue bioassay and two greenhouse trials during 2010–2011. The five hybrids included two non-Bt corn and three Bt corn hybrids representing three transgenic technologies: YieldGard® Corn Borer, Genuity® VT Triple Pro™, and Genuity® SmartStax™. YieldGard® Corn Borer corn contains a single Bt protein (Cry1Ab), while Genuity® VT Triple Pro™ and SmartStax™ contain two and three pyramided Cry genes targeting above-ground lepidopteran pests, respectively. An average of 75.5% of larvae survived after 6 d on non-Bt corn leaf tissue. The 6-d survivorship on Cry1Ab corn leaf tissue was 3% for Cry1Ab-SS, 19% for Cry1Ab-RS, and 35% for Cry1Ab-RR larvae, while none of the three insect genotypes survived for 6-d on the leaf tissue of the two pyramided Bt corn hybrids. After 21 d on whole plants in the greenhouse, 42.6–62.5% of larvae survived on non-Bt corn plants. Larval survivorship rates on YieldGard® Corn Borer plants after 21 d in the greenhouse were 4.7–5.6% for Cry1Ab-SS, 29.4–32.5 % for Cry1Ab-RS, and 36.6–45.6% for Cry1Ab-RR. Both pyramided Bt corn hybrids were very effective against *D. saccharalis* regardless of the insect genotype with 21-d survivorship of <2% for Cry1Ab-SS and Cry1Ab- RS, and <5% for Cry1Ab-RR. Cry1Ab-RS and -RR caused significant entry/exit holes and tunneling inside the plant stalks of non-Bt and YieldGard® corn plants, while they caused little injury on the two pyramided Bt corn hybrids. The data generated from both the leaf tissue and whole plant tests showed that the Cry1Ab-resistant *D. saccharalis* was susceptible to the pyramided Bt corn hybrids. The results suggest that the pyramided Bt corn containing Genuity® VT Triple Pro™ or SmartStax™ traits should offer a means for Cry1Ab resistance management in *D. saccharalis*.

Keywords: Transgenic crop, Resistance management, *Bacillus thuringiensis,* Gene-pyramiding, *Diatraea saccharalis,* Corn borer

1. Introduction

The sugarcane borer, *Diatraea saccharalis* (F), is a major target species of transgenic corn (*Zea mays* L.) expressing *Bacillus thuringiensis* (Bt) proteins in South America and the mid-southern region of the U.S. (PRNewswire, 2009). Initially a major pest of sugarcane, *D. saccharalis* has expanded its host and geographic range to other grasses in the family Poaceae. In many areas of the U.S. gulf coast region, it has recently replaced the southwestern corn borer, *Diatraea grandiosella* (Dyar), as the dominant corn borer species (Reagan, 2001; Castro et al., 2004; Porter et al., 2005; Huang et al., 2012). A field survey from 2004 to 2008 indicated that *D. saccharalis* represented >80% of the total corn borer populations in the major corn planting areas in Louisiana (Huang and Leonard, 2008; Huang et al., 2009).

Since 1999, transgenic corn expressing Bt proteins has been successfully used for management of a complex of corn stalk borers including *D. saccharalis* in the U.S. mid-southern region

(Davis et al., 1999). Resistance development in target insect species is a major concern for the sustainable use of Bt crops (Ostlie et al., 1997; US EPA, 2001). To date, field resistance in target insect species to Bt crops that results in control failure or reduced control efficacy has been documented in at least four cases including resistance of fall armyworm, *Spodoptera frugiperda* (J.E. smith), to Cry1F corn in Puerto Rico (Storer et al., 2010), resistance of African stem borer, *Busseola fusca* (Fuller), to Cry1Ab corn in South Africa (van Rensburg, 2007), resistance of pink bollworm, *Pectinophora gossypiella* (Saunders), to Cry1Ac cotton (*Gossypium hirsutum* L.) in western India (Dhurua and Gujar, 2011), and resistance of western corn rootworm, *Diabrotica virgifera* LeConte, to Cry3Bb1 corn in Iowa, U.S. (Gassmann et al., 2011).

A previous study reported that some of the single gene Bt corn hybrids (e.g. Cry1Ab corn) that was commonly planted in the mid-southern region did not express a high dose against *D. saccharalis* as desired for the "high dose/structured refuge" IRM strategy (Wu et al., 2007). Recently, Ghimire et al. (2011) evaluated six other Bt corn hybrids including four Cry1Ab (YieldGard® Corn Borer) and two Cry1F (Herculex I®) corn hybrids against Cry1Ab-susceptible, -resistant, and -heterozygous genotypes of *D. saccharalis*. The results showed that all six Cry1Ab corn hybrids did not express a high dose for *D. saccharalis*. However, the Cry1Ab-resistant strain of *D. saccharalis* could not survive on two experimental corn lines (event MON89034) containing the pyramided Bt genes for Cry1A.105 and Cry2Ab2 (Ghimire et al., 2011). Gene-pyramiding is a strategy employed to develop transgenic plants that express multiple Bt proteins targeting the same group of insect pests. The first two commercialized pyramided Bt corn technologies in the U.S. for managing lepidopteran pests include Genuity® VT Triple Pro™ and Genuity® SmartStax™. Both were first commercially planted during the 2010 crop season (Monsanto, 2012; US EPA, 2010). The objective of this study was to evaluate larval survival and plant injury of Cry1Ab-susceptible, -resistant, and -heterozygous genotypes of *D. saccharalis* on commercial corn hybrids containing single and pyramided Bt genes and thus to determine if the novel pyramided Bt corn hybrids could overcome the Cry1Ab resistance in *D. saccharalis*.

2. Materials and methods

2.1. Insect sources

Three genotypes of *D. saccharalis* were tested in this study: Cry1Ab-susceptible (Cry1Ab-SS), Cry1Ab-resistant (Cry1Ab-RR), and F1 heterozygous (Cry1Ab-RS) genotypes. The Cry1Ab-SS strain was established from larvae collected from non-Bt plants near Winnsboro in Franklin Parish in northeast Louisiana (32_ 80 600N, 91_ 410 1800) in 2009. The Cry1Ab-SS strain is susceptible to purified Cry1Aa, Cry1Ab, Cry1Ac, Cry1A.105, and Cry2Ab2 proteins (Huang et al., 2007b; Wu et al., 2009), as well as to Bt corn leaf tissue expressing Cry1Ab, Cry1A.105, and Cry2Ab2 (Huang et al., 2011). The Cry1Ab-resistant strain was obtained from a single two-parent family developed through an F2 screen in 2004 (Huang et al., 2007a). The resistant strain can survive and complete its entire larval development (from neonate to pupa) on commercial Cry1Ab corn plants in the greenhouse (Huang et al., 2007a). Before the Cry1Ab-RR strain was used in this study, it had been backcrossed with the Cry1Ab-SS strain two times and reselected for Cry1Ab resistance on Cry1Ab corn leaf tissue in the F2 generations. The Cry1Ab-RS was produced from a cross between Cry1Ab-SS and the backcrossed-reselected Cry1Ab-RR.

2.2. Corn hybrids

Three Bt and two non-Bt commercial corn hybrids from Monsanto Company (St. Louis, MO) were evaluated during 2010 and 2011. The three Bt corn hybrids were DKC 67-23 (YGCB) containing the YieldGard® Corn Borer trait, DKC 67-88 (VT3P) containing the Genuity® VT Triple Pro™ traits and DKC 61-21(SMT) containing the Genuity® SmartStax™ traits. YieldGard® Corn Borer contains a single Bt protein, Cry1Ab. The pyramided Bt corn hybrids were recently approved for planting; previously, YieldGard® corn was the most commonly planted Bt corn technology for corn stalk borer control in the U.S., including the mid-southern region. Genuity® VT Triple PRO_ is a pyramided Bt corn that expresses three Bt genes including Cry1A.105 and Cry2Ab2 for controlling above-ground lepidopteran pests and Cry3Bb1 for managing underground rootworms, *Diabrotica* spp. Genuity® SmartStax™ corn contains all Bt genes expressed in Genuity® VT Triple Pro™ as well as Cry1F targeting lepidopteran species and Cry34Ab1/Cry35Ab1 targeting rootworms (Gatehouse, 2008; Monsanto, 2012). The two non-Bt corn hybrids were DKC 61-22 (Non-BtS) and DKC 67-86 (Non- BtY). The hybrid, DKC 61-22, was genetically closely related to the Bt corn hybrid, DKC 61-21, while DKC 67-86 was closely related to the Bt corn hybrids DKC 67-23 and DKC 67-88.

Two seeds of a hybrid were planted in each 18.9-L plastic pot which contained ~5 kg of standard potting soil mixture (Perfect Mix™, Expert Gardener products, St. Louis, MO) in a greenhouse located in Baton Rouge, LA. The pots were kept on tables in the greenhouse allowing an approximately 1-m distance between plot to plot and 20-cm distance between pot to pot. Seed planting and plant management procedures in the greenhouse were similar to those described in Wu et al. (2007). Fertilization, topping-up, and irrigation among other management practices were used when needed to ensure optimum growth. Expression of Cry proteins for a corn hybrid was confirmed using ELISA-based assays for two plants/treatment that were randomly selected from the plots (EnviroLogix, Quantiplate™ kits, Portland, ME).

2.3. Leaf tissue bioassay

In 2011, fully expanded leaves at V6eV8 stages of corn (Ritchie et al., 1993) were removed from greenhouse grown plants and used in the laboratory bioassays. Leaf tissue bioassays were carried out in the laboratory following methods similar to those described in Huang et al. (2006) and Ghimire et al. (2011). In the bioassay, six pieces of leaf tissue (~20 cm^2) of a corn hybrid were placed in each well of 8-well trays (Bio-Smart-8, C-D International, Pitman, NJ). Twenty-five neonates of each of the three genotypes of *D. saccharalis* were then placed in each well. Bioassay trays were placed in a growth chamber maintained at 28 °C, a 14:10 L: D cycle and humidity of 40–50%. Leaf tissues were replaced with fresh tissues after 3 d. Larval survival was recorded on the 6th d after release of neonates. There were four replications (n = 100) for each combination of corn hybrid and insect genotype.

2.4. Whole plant tests in greenhouse

Two independent trials were conducted to evaluate larval survival and plant injury of three genotypes of *D. saccharalis* on whole plants in the greenhouse. In each trial, 20 (trial one in 2010) or 10 (trial two in 2011) neonates (<24 h old) of each of the three insect genotypes were placed manually into the collar of the leaf directly above or below the uppermost ear at the reproductive plant stages (R1–R2) (Ritchie et al., 1993) using a soft brush (size 10/0; DalereRowney Ltd., Bracknell, England). Each treatment combination of corn hybrid and insect geno-

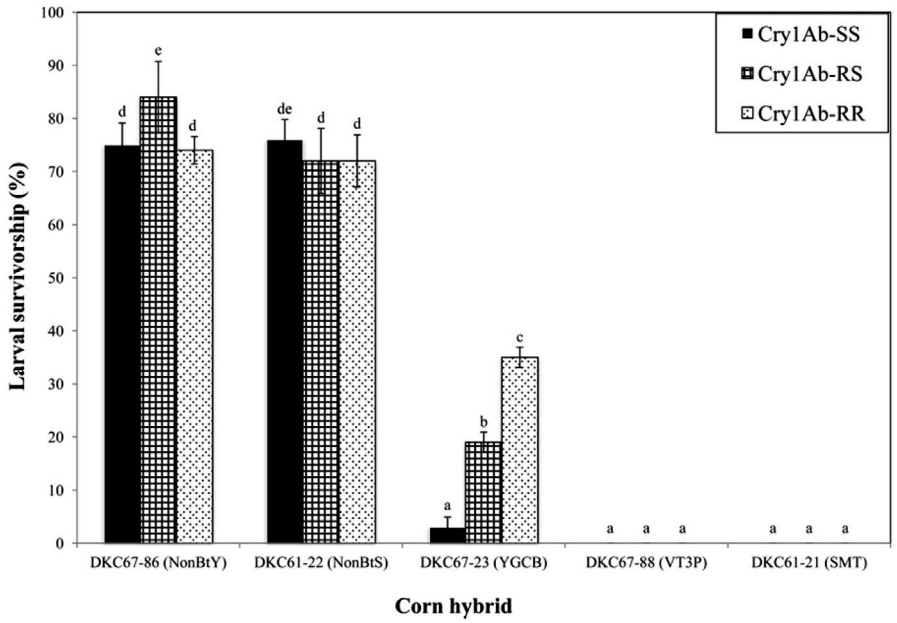

Figure 1. Larval survivorship (% mean ± sem) of Cry1Ab-susceptible (Cry1Ab-SS), -heterozygous (Cry1Ab-RS), and -resistant (Cry1Ab-RR) genotypes of *Diatraea saccharalis* after 6 d on leaf tissue of two non-Bt and three Bt corn hybrids containing single or multiple Cry proteins. Mean values followed by the same letter are not significantly different (α > 0.05; SAS PROC GLM, LSMEANS test).

type was replicated four times in a randomized complete block design in each trial. There were four plants (two pots, each with two plants) for each replication. The number of surviving insects on each plant, number of entry/exit holes, and tunnel length inside each stalk were recorded 21 d after larval release.

2.5. Data analysis

Larval survival recorded on leaf tissues in the laboratory bioassay and on whole plants in the greenhouse tests were transformed using arcsine ($x^{0.5}$) to normalize the treatment variances, while the number of entry/exit holes and tunnel length inside stalks were log(x + 1) transformed (Zar, 1984). The transformed data then were analyzed using two-way ANOVA (SAS Institute, 2010) with insect genotype and corn hybrid as the two main factors. Treatment differences were determined using LSMEANS tests (SAS Procedure GLM) at α = 0.05 level of significance (SAS Institute, 2010). The untransformed data and standard errors of the means (SEM) are presented in the figures. Additionally, the effective dominance level (D_{ML}) for Cry1Ab resistance in *D. saccharalis* was calculated using the formula as described in Bourguet et al. (2000).

3. Results

3.1. Larval survival of Cry1Ab-SS, Cry1Ab-RS, and Cry1Ab-RR genotypes of D. saccharalis on leaf tissues of two non-Bt and three Bt corn hybrids

The effects of corn hybrid, insect genotype, and their interaction on 6-d larval survivorship of *D. saccharalis* were significant for all factors (F = 454.8; df = 4, 42; P < 0.0001 for corn hybrid; F = 6.74; df = 2, 42; P = 0.0029 for insect genotype; and F = 8.07; df = 8, 42; P < 0.0001 for interaction). Larval survival of *D. saccharalis* on non- Bt leaf tissue was in general not significantly different on non-Bt corn plants across the three insect genotypes with an average survivorship of 75.5% after 6 d (Figure 1). Larval survivorship of the three insect genotypes on Bt corn leaf tissue was significantly (P < 0.05) less than that on non-Bt corn leaf tissue. Only very low survivorship (3%) of Cry1Ab-SS larvae was observed after 6 d on leaf tissue of YieldGard® plants. However, 35% larvae of Cry1Ab-RR survived on YieldGard® corn leaf tissue, which was significantly greater than that for Cry1Ab-SS. In addition, an average of 19% larvae of Cry1Ab-RS genotype also survived after 6 d on YieldGard® corn leaf tissue, which was significantly (P < 0.05) less than that of Cry1Ab-RR but significantly (P < 0.05) greater than that of Cry1Ab-SS. Both pyramided Bt corn hybrids provided excellent control of *D. saccharalis*. All larvae were killed after 6 d on leaf tissue removed from the two pyramided Bt corn hybrids regardless of the insect genotype (Figure 1).

3.2. Larval survival and plant injury of Cry1Ab-SS, -RS, and -RR genotypes of D. saccharalis on whole plants of two non-Bt and three Bt corn hybrids: Trial one—2010

In the first greenhouse trial, which was conducted in 2010, the main effect of corn hybrid on larval survivorship of *D. saccharalis* after 21 d on whole plants was significant (F = 111.35; df = 4, 42; P < 0.0001). The effect of insect genotype and the interaction of corn hybrid and insect genotype were also significant (F = 11.43; df = 2, 42; P = 0.0001 for insect genotype and F = 2.76; df = 8, 42; P = 0.0153 for interaction). Larval survivorship on the two non-Bt corn hybrids ranged from 42.6 to 56.9% and was not significantly (P > 0.05) different across the three insect genotypes (Figure 2). Cry1Ab-SS was susceptible to YieldGard® corn plants with only 4.7% survival after 21 d. Both Cry1Ab-RR and Cry1Ab-RS larvae survived well on Cry1Ab corn plants with 21-d survivorship of 36.6 and 29.4%, respectively. The survivorship rates of Cry1Ab-RR and -RS larvae observed on YieldGard® plants were not significantly (P < 0.05) different from those recorded on non-Bt plants. As observed in the leaf tissue tests, both pyramided Bt corn hybrids were very effective against all three insect genotypes. Larval survivorship of the three insect genotypes on the two pyramided Bt corn hybrids was low, <5%, and there were no significant differences on the two corn hybrids across the three insect genotypes (Figure 2).

Effects of corn hybrid, insect genotype, and their interaction on number of entry/exit holes counted on stalks were also significant (F = 166.21; df = 4, 42; P < 0.0001 for corn hybrid, F =

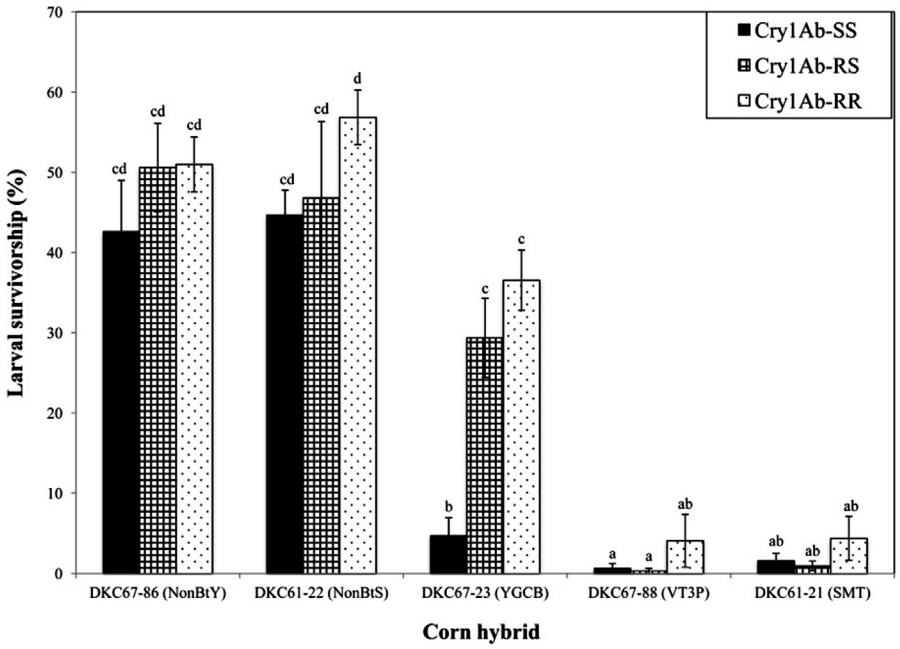

Figure 2. Larval survivorship (% mean ± sem) of Cry1Ab-susceptible (Cry1Ab-SS), -heterozygous (Cry1Ab-RS), and -resistant (Cry1Ab-RR) genotypes of *Diatraea saccharalis* after 21 d on whole plants of two non-Bt and three Bt corn hybrids containing single or multiple Cry proteins (First trial-2010). Mean values followed by the same letter are not significantly different (α > 0.05; SAS PROC GLM, LSMEANS test).

13.08; df = 2, 42; $P < 0.0001$ for insect genotype, and $F = 3.32$; df = 8, 42; $P = 0.0049$ for interaction). The number of entry/exit holes did not significantly differ ($P > 0.05$) on the two non-Bt corn hybrids across all the insect genotypes with an average of 17.9 holes/stalk (Figure 3a). Cry1Ab-SS larvae on YieldGard® plants made significantly fewer holes on the stalks with an average of 2.1 holes/stalk. In contrast, Cry1Ab-RR and -RS larvae on YieldGard® plants caused an average of 11 and 10.2 holes/stalk, respectively. The number of holes produced by Cry1Ab-RR on YieldGard® plants was not significantly different from those

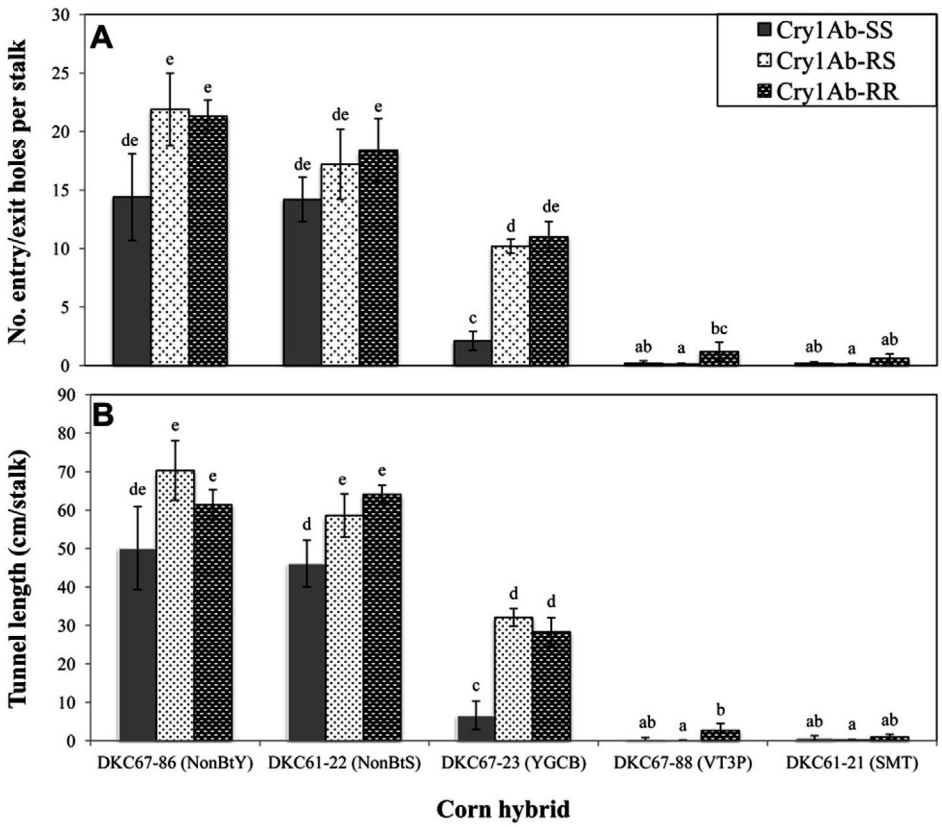

Figure 3. Number of entry/exit holes (A: mean ± sem) and stalk tunnel length (B: cm, mean ± sem) of Cry1Ab-susceptible (Cry1Ab-SS), -heterozygous (Cry1Ab-RS), and –resistant (Cry1Ab-RR) genotypes of *Diatraea saccharalis* after 21 d on whole plant of two non-Bt and three Bt corn hybrids containing single or multiple Cry proteins (First trial-2010). Mean values followed by the same letter are not significantly different (α > 0.05; SAS PROC GLM, LSMEANS test).

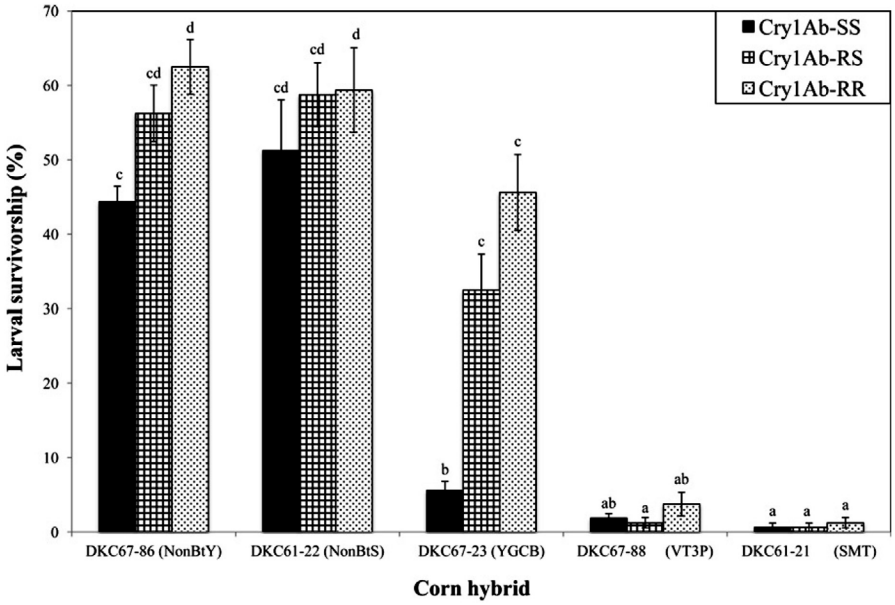

Figure 4. Larval survivorship (% mean ± sem) of Cry1Ab-susceptible (Cry1Ab-SS), -heterozygous (Cry1Ab-RS), and -resistant (Cry1Ab-RR) genotypes of *Diatraea saccharalis* after 21 d on whole plants of two non-Bt and three Bt corn hybrids containing single or multiple Cry proteins (Second trial-2011). Mean values followed by the same letter are not significantly different (α > 0.05; SAS PROC GLM, LSMEANS test).

of the three insect genotypes on the two non- Bt corn hybrids. The number of holes on YieldGard® plants was also not significant between Cry1Ab-RS and Cry1Ab-RR larvae. As observed in larval survivorship, both pyramided Bt corn hybrids were very effective in reducing stalk boring of *D. saccharalis* regardless of the insect genotype. The number of holes bored by the three insect genotypes was low, ranging from 0.1 to 1.2 holes/stalk, and there were no significant differences on the two pyramided Bt corn hybrids across the three insect genotypes (Figure 3a).

Effects of corn hybrids, insect genotypes, and their interactions on stalk tunnel length caused by *D. saccharalis* after 21 d were also significant (F = 186.59; df = 4, 42; P < 0.0001 for corn hybrid, F = 9.02; df = 2, 42; P = 0.0006 for insect genotype, and F = 3.5; df = 8, 42; P = 0.0035 for interaction). Stalk tunnel length by the three insect genotypes on the five corn hybrids was highly correlated to the larval survivorship and the number of entry/exit holes. The tunnel length on two non-Bt corn hybrids was not significantly different (P > 0.05) and ranged from 46.1 to 70.3 cm/stalk across the three insect genotypes. Cry1Ab-SS larvae on YieldGard® plants caused an average of 6.6 cm tunnel length per stalk, which was significantly shorter (P < 0.05) than those observed on non-Bt plants. Both Cry1Ab-RR and -RS larvae caused significant stalk injury on YieldGard® plants with an average tunnel length of 28.3 and 32.1 cm/stalk, respectively (Figure 3b). The tunnel length on YieldGard® caused by Cry1Ab-RS was not significantly different from those observed on the non-Bt plants, and the tunnel length made by Cry1Ab-RR was also not significantly different from those observed on the non-Bt corn plants infested with Cry1Ab-SS larvae. However, both pyramided Bt corn hybrids were highly effective in reducing stalk tunneling of *D. saccharalis* regardless of the insect genotype. Tunnel length per stalk on the two pyramided Bt corn hybrids ranged from only 0.1–2.7 cm across the three insect genotypes, which was even significantly shorter than that (6.6 cm) of Cry1Ab-SS on YieldGard® plants. The tunnel length (2.7 cm) of Cry1Ab-RR on Genuity® VT Triple Pro™ hybrid was statistically significantly greater than those (0.1–0.2 cm) of Cry1Ab-RS larvae on the two pyramided Bt corn hybrids, but the differences were small (Figure 3b).

3.3. Larval survival and plant injury of Cry1Ab-SS, -RS, and -RR genotypes of D. saccharalis on whole plants of two non-Bt and three Bt corn hybrids: Trial two—2011

The overall performance of the three genotypes of *D. saccharalis* on the five corn hybrids was consistent in the two trials conducted in 2010 and 2011. Larval survival of *D. saccharalis* after 21 d in the trial performed in 2011 was also significantly affected by corn hybrid (F = 194.98; df = 4, 42; P < 0.0001), insect genotype (F = 17.0; df = 2, 42; P < 0.0001), and their interaction (F = 5.01; df = 8, 42; P = 0.0002) (Figure 4). Larval survivorship on the two non-Bt corn hybrids ranged from 43.4 to 62.5% and was not significantly different (P > 0.05) across the three insect genotypes. Survivorship of Cry1Ab-RR and -RS on YieldGard® plants was 45.6 and 32.5%, respectively, which was significantly greater than that (5.6%) of Cry1Ab-SS but was not significantly different from most of those observed on the two non-Bt plants. Again, both pyramided Bt corn hybrids were very effective against all three insect genotypes. Larval survivorships (0.6e3.8%) on the two pyramided Bt corn hybrids was not significantly different on the two hybrids across the three insect genotypes (Figure 4).

Data on the number of entry/exit holes recorded in the 2011 trial were also consistent with those observed in the 2010 trial. The main effect of corn hybrid and insect genotype on number of entry/ exit holes was significant (F = 337.06; df = 4, 42; P < 0.0001 for corn hybrid and F = 31.22; df = 2, 42; P < 0.0001 for insect genotype). The interaction of corn hybrid and insect genotype was also significant (F = 8.39; df = 8, 42; P < 0.0001). The number of entry/exit holes on non-Bt corn plants ranged from 9.5 to 12.9 and the number was not significantly different on the two hybrids across the three insect genotypes (Figure 5a). An average of 7.5 and 5.3 holes/stalk were observed on YieldGard® plants that were infested with Cry1Ab-RR and Cry1Ab-RS, respectively. The number of holes on YieldGard® plants caused by Cry1Ab-RR or Cry1Ab-RS was significantly less than those observed on the two non-Bt corn plants but was significantly greater than those (0.9 holes/stalk) made by Cry1Ab-SS larvae. Again, both pyramided Bt corn hybrids were effective in reducing the number of holes caused by *D. saccharalis* regardless of

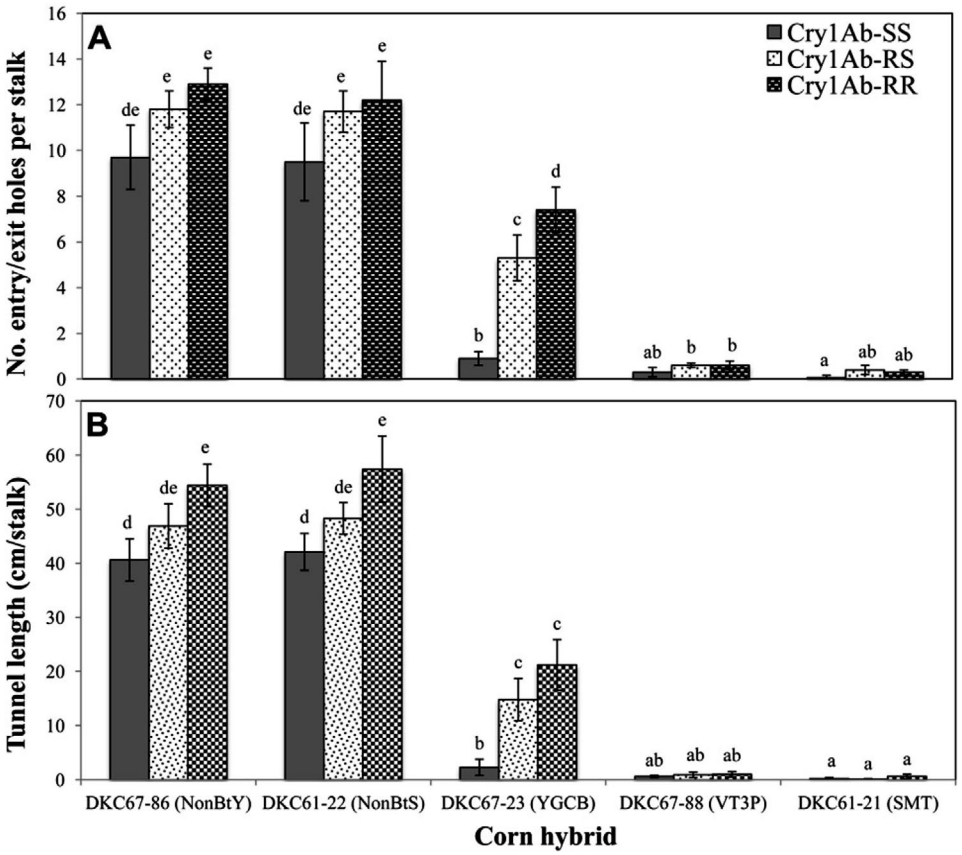

Figure 5. Number of entry/exit holes (A: mean ± sem) and stalk tunnel length (B: cm, mean ± sem) of Cry1Ab-susceptible (Cry1Ab-SS), -heterozygous (Cry1Ab-RS), and –resistant (Cry1Ab-RR) genotypes of *Diatraea saccharalis* after 21 d on whole plants of two non-Bt and three Bt corn hybrids containing single or multiple Cry proteins (Second trial-2011). Mean values followed by the same letter are not significantly different (α > 0.05; SAS PROC GLM, LSMEANS test).

the insect genotype. The number of entry/exit holes on the two pyramided corn hybrids was <1 per stalk and was in general not significantly different on the two corn hybrids across the three insect genotypes (Figure 5a).

As observed in the first trial, the tunnel length in stalks of the five corn hybrids across the three insect genotypes was highly correlated with the larval survival and number of entry/exit holes on the stalks. Again, effect of corn hybrid, insect genotype, and the interaction on tunnel length was significant (F = 250.28; df = 4, 42; P < 0.0001 for corn hybrid, F = 13.38; df = 2, 42; P < 0.0001 for insect genotype, and F = 5.46; df = 8, 42; P < 0.0001 for interaction). Tunnel length on the two non-Bt corn hybrids ranged from 40.6 to 57.4 cm/stalk and was not significantly different (P > 0.05) across the three insect genotypes. Larvae of Cry1Ab-RR and -RS on YieldGard® plants caused an average tunnel length of 21.2 and 14.8 cm/stalk, respectively, which was significantly (P < 0.05) shorter than those of the three insect genotypes on non-Bt plants, but was significantly (P < 0.05) longer than that (2.3 cm/stalk) of Cry1Ab-SS on YieldGard® plants. In contrast, larvae of *D. saccharalis* caused only very short tunnels, ≤1 cm/stalk, on the two pyramided Bt corn hybrids regardless of the insect genotypes (Figure 5b).

3.4. Effective dominance level (DML) of Cry1Ab resistance in D. saccharalis

Survivorship of Cry1Ab-RR was 0% on leaf tissue of the two pyramided Bt corn hybrids after 6 d and only 1.25–4.3% on plants after 21 d. The results showed that Cry1Ab-resistant larvae of *D. saccharalis* were virtually not resistant to the two pyramided Bt corn hybrids. For this reason, effective dominance level, DML, of Cry1Ab resistance in *D. saccharalis* could be calculated only for the tests with the YieldGard® Corn Borer hybrid. The DML value was 0.5 based on the 6-d larval survivorship on the leaf tissue test and 0.67–0.78 on the whole plant tests in the greenhouse (Table 1). The results suggest that Cry1Ab resistance in *D. saccharalis* was functionally incompletely dominant on Cry1Ab corn leaf tissue and whole Cry1Ab corn plants.

4. Discussion

Data on larval survival and plant injury showed that the three insect genotypes of *D. saccharalis* were effective in establishing themselves on the two non-Bt corn plants. The larval survivorship (72–84% on leaf tissue after 6 d and 42.6–62.5% on intact plants after 21 d) observed in the current study was similar to that reported in other earlier studies (Walker et al., 2000;Wu et al., 2007; Ghimire et al., 2011). Larvae of all three genotypes of *D. saccharalis* on non-Bt corn plants also made a substantial number of entry/exit holes on the stalks and caused significant tunneling inside stalks. The results suggest that the artificial diet and leaf tissue selection to maintain the insect strains in the

Table 1. Effective dominance level (D_{ML}) of Cry1Ab resistance in *Diatraea saccharalis* on Cry1Ab corn leaf tissue and intact Cry1Ab corn plants.

Trial	Corn hybrid	D_{ML}
Leaf tissue bioassay	YieldGard®	0.50
Whole plants in 2010	YieldGard®	0.78
Whole plants in 2011	YieldGard®	0.67

laboratory (Huang et al., 2007a) had not measurably reduced their adaptation to corn plants. As reported in two previous studies (Wu et al., 2007; Ghimire et al., 2011), larvae of the Cry1Ab-resistant genotype of *D. saccharalis* in the current study demonstrated high survivorship on both leaf tissue and whole plants of YieldGard® plants expressing the Cry1Ab protein. The results again confirmed that the Cry1Ab-RR genotype of *D. saccharalis* was highly resistant to Cry1Ab corn plants.

To delay resistance development, a "high dose/structured refuge" strategy has been adopted for planting the first generation Bt corn that expresses a single Bt protein (e.g. YieldGard® Bt corn). One of the key assumptions for the "high dose/refuge" strategy is that resistance in the target species should be recessive so that a high percentage of resistant heterozygotes can be killed by "high dose" expressed Bt corn (Andow and Hutchison, 1998; US EPA, 2001; Bourguet et al., 2003). However, both the leaf tissue bioassays and whole plant tests showed significant survivorship of Cry1Ab-RS genotype. These results suggest that Cry1Ab resistance in *D. saccharalis*, rather than being recessive, was functionally incompletely dominant on the Cry1Ab corn hybrid tested in this study. The estimated effective dominance level (0.5–0.78) also suggest that the Cry1Ab corn hybrid did not express a "high dose" as defined in the "high dose/refuge" strategy for *D. saccharalis*. Several other Cry1Ab corn hybrids evaluated in two previous studies (Wu et al., 2007; Ghimire et al., 2011) also did not provide a high dose for *D. saccharalis*, especially in the reproductive plant stages.

In spite of the high resistance to Cry1Ab corn, both leaf tissue bioassays and whole plant tests showed that the Cry1Ab-RR and -RS larvae of *D. saccharalis* were susceptible to the two pyramided Bt corn hybrids. Although data generated for this study could not provide sufficient information to determine if the two pyramided Bt corn hybrids produced a "high dose" of Bt proteins for *D. saccharalis* as defined in the "high dose/refuge" strategy, the results of this study provided clear evidence that the novel pyramided Bt corn hybrids containing Genuity® VT Triple PRO_ or SmartStax™ traits are effective against *D. saccharalis* and can overcome the Cry1Ab resistance in *D. saccharalis*. Both Genuity® VT Triple PRO_ and SmartStax™ corn contain the Cry1A.105 and Cry2Ab2 proteins. The Cry1A.105 is a chimeric gene comprised of domains I and II from Cry1Ab and Cry1Ac and domain III of Cry1F (Biosafety Clearing House, 2009). Previous laboratory bioassays showed that the Cry1Ab-resistant strain of *D. saccharalis* demonstrated only a very low level (4.1-fold) of resistance to the Cry1A.105 protein and was equally susceptible to the Cry2Ab2 protein as its Cry1Ab-SS counterpart (Wu et al., 2009). Additionally, laboratory and greenhouse tests with two experimental corn lines containing the Cry1A.105 and Cry2Ab2 proteins also showed that the pyramided Bt corn lines could completely overcome the Cry1Ab resistance in *D. saccharalis* (Ghimire et al., 2011). Together with the previous data, the results showed that the pyramided corn technologies containing the Genuity® VT Triple Pro™ or SmartStax™ traits should provide a means for managing the Cry1Ab resistance in *D. saccharalis*.

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