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Fitness costs and stability of Cry1Ab resistance in sugarcane borer, *Diatraea saccharalis* (F.) ☆

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ABSTRACT

The sugarcane borer, *Diatraea saccharalis* (F.), is a major target species of transgenic corn expressing *Bacillus thuringiensis* (Bt) proteins in South America and the U.S. mid-south region. In this study, the fitness of seven insect genotypes of *D. saccharalis* were assayed on non-toxic diet, which included a Cry1Ab-susceptible strain (SS-2009), two Cry1Ab-resistant strains (RR-43A_{BC}, RR-L5B_{BC}), and four F_1 hybrids (F_1-R43$A_m S_f$, F_1-R43$A_f S_m$, F_1-R5$B_m S_f$, and F_1-R5$B_f S_m$). The F_1 hybrids were generated by reciprocal crosses of SS-2009 with RR-43A_{BC} and RR-L5B_{BC}, respectively. Biological parameters measured were neonate-to-pupa survivorship, neonate-to-pupa development time, pupal mass, pupa-to-adult emergence rate, and progeny (neonates) production. The overall performance of the two resistant strains and the four F_1 genotypes was either similar or better than SS-2009 for all biological parameters measured, suggesting a lack of fitness costs associated with the Cry1Ab resistance traits in both RR-43A_{BC} and RR-L5B_{BC}. In addition, resistance stability was evaluated by measuring the Cry1Ab susceptibility of RR-43A_{BC} and RR-L5B_{BC} in the absence of selection pressure. Laboratory bioassays showed that larval mortality of the two resistant strains did not significantly increase after selection pressure was removed for 16 generations across all Cry1Ab concentrations assayed. The results provide valuable information on assessing resistance risk and developing effective management strategies for the sustainable use of Bt corn technology.

1. Introduction

The sugarcane borer, *Diatraea saccharalis* (F.), is a major target species of transgenic corn, *Zea mays* L., containing *Bacillus thuringiensis* (Bt) in South American and the mid-southern region of the United States (Porter et al., 2005; PRNewswire, 2009; Huang et al., 2012). Resistance evolution in field populations of target species is a great threat to the sustainable use of Bt crops (Tabashnik and Carrière, 2007; Matten et al., 2012). Since Bt crops were first commercially planted in 1996, field resistance in target insect species due to extensive use of the technology has been documented in at least four cases in the world (see review in Huang et al., 2011a).

Many factors including fitness costs and stability of Bt resistance can influence resistance evolution in pest populations (Tabashnik and Carrière, 2007; Onstad, 2008; Tabashnik et al., 2008; Head and Greenplate, 2012). Fitness is the ability of an individual of a certain genotype to survive and reproduce relative to other individuals of the same species. A fitness cost of Bt resistant genotype is when the homozygous resistant insects feeding non-Bt plants have lower fitness than their susceptible counterparts on the same non-Bt plants (see review in Gassmann et al., 2009a). Relative to its susceptible counterpart, a resistant population with fitness costs often exhibits higher mortality, delayed growth/development, and lower reproduction in the absence of selection pressures (Carrière et al., 2010). Therefore, fitness costs may act to delay resistance to Bt toxins. Although, fitness costs have not been considered as a factor in most Bt resistance management models, it is believed to have played an important role in maintaining the long-term susceptibility for some species targeted by Bt crops (Huang et al., 2011a).

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The fitness costs and stability of a Cry1Ab-resistant strain of *D. saccharalis* that was isolated from a field population collected in 2004 (hereafter called RR-2004) have been previously evaluated (Wu et al., 2009a; Huang et al., 2011b). The results showed that the Cry1Ab resistance in RR-2004 was stable and not associated with notable fitness costs. RR-2004 was established from a two-parent family collected in a corn field in northwest Louisiana and identified using an F_2 screen (Huang et al., 2007a). It was shown to possess a major resistance allele to commercial Cry1Ab corn (Huang et al., 2007a). During 2009, another intensive screening was conducted to determine the changes in Cry1Ab resistance allele frequency in Louisiana populations of *D. saccharalis*. In that survey, a total of 258 field-collected individuals of *D. saccharalis* were collected from three locations in Louisiana and examined for resistance to Cry1Ab corn plants using the F_2 screen (Huang et al., 2012). Eight of these F_2 families were identified to possess major resistance alleles to Cry1Ab corn plants. Several Cry1Ab-resistant strains corresponding to the eight F_2 families were established in the laboratory (Huang et al., 2012). The main objective of this study was to determine the fitness of Cry1Ab-susceptible, heterozygous, and -resistant genotypes on non-Bt diet using two of the resistant strains of *D. saccharalis* that had shown the highest resistance levels to Cry1Ab (Zhang et al., 2013). In addition, stability of the two Cry1Ab-resistant strains was also evaluated in the absence of selection. The results should provide valuable information on assessing Bt resistance risk in the corn borer populations.

2. Materials and methods

2.1. Cry1Ab protein

Purified (99.9%) Cry1Ab protein was obtained from Case Western Reserve University, Ohio. The Cry proteins were produced using recombinant *Escherichia coli* culture and were subsequently activated with trypsin (Pusztai-Carey et al., 1995). The trypsin-activated Cry proteins were lyophilized and stored at −80 °C. The protein purity was determined using high-performance liquid chromatography and sodium dodecyl sulfate polyacrylamide gel electrophoresis (Pusztai-Carey et al., 1995; Masson et al., 1998).

2.2. Insect sources

A Cry1Ab-susceptible strain (SS-2009) and two Cry1Ab-resistant strains (RR-43A_{BC} and RR-L5B_{BC}) of *D. saccharalis* were used as the insect sources in this study. SS-2009 was established from larvae collected from non-Bt corn fields near Winnsboro in Northeast Louisiana during 2009. It has been documented to be susceptible to purified Cry1Ab, Cry1Aa, Cry1Ac, and Cry1F proteins as well as to Cry1Ab-corn, Cry1F-corn, Genuity VT Triple Pro, and SmartStax Bt corn hybrids (Huang et al., 2012; Wangila et al., 2012; Zhang et al., 2013). RR-43A_{BC} and RR-L5B_{BC} originated from two of the eight Cry1Ab-resistant strains of *D. saccharalis* that were derived from the corresponding eight two-parent families collected in 2009 at the same location as SS-2009 (Huang et al., 2012). The two parents of RR-43A_{BC} were collected from non-Bt corn plants, while one of the two parents of RR-L5B_{BC} was collected from a Bt corn plant (Huang et al., 2008). All of the eight families were identified to carry major resistance alleles to commercial Cry1Ab corn hybrids (e.g. YieldGard® Corn Borer) by using an F_2 screening method (Huang et al., 2012). Among these, RR-43A_{BC} and RR-L5B_{BC} demonstrated the highest levels (>526-flod) of resistance to Cry1Ab protein in a previous study (Zhang et al., 2013). Laboratory studies also showed that both RR-43A_{BC} and RR-L5B_{BC} exhibited an equivalent survivorship on Cry1Ab corn leaf tissue as our previously documented Cry1Ab-resistant strain (RR-2004) (Huang et al., 2012). RR-2004 was established in 2004 also using the F_2 screen. Greenhouse studies showed that larvae of RR-2004, RR-43A, and RR-L5B were able to complete development on commercial Cry1Ab corn hybrids (Huang et al., 2007b, 2012). Individuals of RR-43A_{BC} and RR-L5B_{BC} had been backcrossed twice with the SS-2009 colony and re-selected for Bt resistance with Cry1Ab corn leaf tissue in the F_2 generations of the backcrosses using a similar selection method as the F_2 screen (Huang et al., 2007c). The original SS-2009 and the two backcrossed and re-selected populations (RR-43A_{BC} and RR-L5B_{BC}) were used in both the fitness and resistance stability studies. In addition, four F_1 genotypes that were developed from the reciprocal crosses between SS-2009 and RR-43A_{BC} (F_1-R43A_mS_f and F_1-R43A_fS_m) and the reciprocal crosses between SS-2009 and RR-L5B_{BC} (F_1-RL5B_mS_f and F_1-RL5B_fS_m) were also included in the fitness tests.

To evaluate the resistance stability in absence of selection, the backcrossed and re-selected RR-43A_{BC} and RR-L5B_{BC} strains were divided into two sub-strains. The two original resistant strains (RR-43A_{BC} and RR-L5B_{BC}) were maintained under selections on Cry1Ab corn leaf tissue as described in the F_2 screen (Huang et al., 2007c), while the other two (RR-43A-UNsel that was generated from RR-43A_{BC} and RR-L5B-UNsel that was derived from RR-L5B_{BC}) were reared on non-Bt corn leaf tissue/meridic diet for 16 generations in the absence of selection pressures. Cry1Ab susceptibility of SS-2009, RR-43A_{BC}, RR-L5B_{BC}, RR-43A-UNsel, and RR-L5B-UNsel was monitored at various generations using a diet-incorporated bioassay as described below.

2.3. Survival, growth, and development in fitness tests

To assess the fitness costs of the Cry1Ab resistance in *D. saccharalis*, two independent sets of bioassays were conducted on non-toxic diet (Bio-Serv, Frenchtown, NJ) at two different times, one with RR-43A_{BC}, SS-2009, F_1-R43A_mS_f, and F_1-R43A_fS_m (hereafter called Assay-43A_{BC}) and another with RR-L5B_{BC}, SS-2009, F_1-RL5B_mS_f, and F_1-RL5B_fS_m (hereafter called Assay-L5B_{BC}). In the bioassays, approximately 1 g of the meridic diet was placed into each cell of the 128-cell trays (Bio-Ba-128, C-D International Inc. Pitman, NJ) using 20-ml syringes (Becton, Dickinson and Company, Franklin Lakes, NJ) as described in Wu et al. (2009a). One neonate (<24 h old) was placed on the diet surface in each cell. A randomized complete block design (RCB) was used in each bioassay. There were four replications (blocks) for each insect genotype with 30 larvae in each replication (n = 120 for each insect genotype). Bioassay trays within a block were held together and placed in a growth chamber maintained at 28 °C, ~50% RH, and a photoperiod of 16:8 (L:D) h. After 7 days, survivors were transferred into 30-ml cups (SOLO, Chicago, IL, USA) containing approximately 5 ml of diet (1 larva/cup) and were allowed to develop to the pupal stage. After the first pupa was observed, all cups were monitored daily until all insects pupated or died. Neonate-to-pupa survival, neonate-to-pupa development time, pupal mass, and moth emergence rate were calculated for each insect genotype.

2.4. Progeny production in fitness tests

Pupae collected from the above rearing cups were separated by sex for each genotype. A pair of newly emerged (<24 h old) virgin male and female adults were placed into each 450-ml paper container (Huhtamaki Foodservice, De Soto, Kansas) containing approximately 10 g of vermiculite (Sun Gro, Pine Bluff, AR) as described in Huang et al. (2007c). A RCB design was used in the progeny production study. The adult containers for each replication (block) were held in a 69-L plastic container (Sterillite, Townsend, MA) to maintain a relatively high RH, which was placed in an environmental chamber at 28 °C, >90% RH, and a photoperiod of 14:10 (L:D) h. For each insect genotype, there were four replications with