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Susceptibility of *Helicoverpa armigera* from different host plants in northern China to *Bacillus thuringiensis* toxin Cry1Ac

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ABSTRACT

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is a serious pest of cotton and many other crops in northern China. To evaluate the contribution of alternative hosts as an effective refuge for transgenic cotton expressing the *Bacillus thuringiensis* (Bt) Cry1Ac toxin, the susceptibility to this toxin was measured in progeny derived from field-collected *H. armigera* larvae and pupae from different hosts in the Xiajin's region of the Shandong Province in northern China. During 2008–2010, progeny from a total of 258,56,184 and 160 single-pair crosses derived from wheat (first-generation), Bt cotton (second-generation), Bt cotton (third-generation), and corn (third-generation) were screened on Cry1Ac diets, respectively. Based on relative average development rates (RADR) of *H. armigera* larvae in these F_1 tests, the second and third-generation moths emerging from Bt cotton fields were more tolerant to the Bt toxin than the first and third-generation moths emerging from wheat and corn each year. These results suggest that there is significant variation in susceptibility to Bt toxins among *H. armigera* populations derived from different host crops. Alternate crops, such as corn, that maintain Bt susceptible populations of *H. armigera* could be used as refugia to minimize the evolution of resistance to Bt cotton.

1. Introduction

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) is a serious pest of cotton, corn, sorghum, and many other crops in northern China (Wu and Guo, 2005; Wu, 2007). Transgenic insecticidal cotton (*Gossypium hirsutum* L.) producing the Cry1Ac protein from the soil bacterium *Bacillus thuringiensis* (Bt cotton) has been effective in controlling this insect pest since 1997. Since 2000, Bt cotton has almost completely replaced non-transgenic cotton cultivars (Wu and Guo, 2005). Bt cotton expressing Cry1Ac has been widely successful in reducing the use of synthetic insecticides (Guo, 1998; Huang et al., 2002; Wu and Guo, 2005; Wu et al., 2008). However, several cases of tolerance to Bt crops have been detected in the United States, Puerto Rico and South Africa (Tabashnik et al., 2009; Blanco et al., 2010; Storer et al., 2010; Bagla, 2010).

Although several approaches have been proposed for delaying pest resistance to Bt crops, the refuge strategy has been the primary approach used in the field. Because the small size of farms and cotton fields typical of China may allow non-cotton crops to act as refugia and other factors, China does not mandate the conventional cotton refugia strategy for the insecticide resistance management that the US and Australia do (Gould, 1998; Shelton et al., 2002; Tabashnik et al., 2005; Wu, 2007). In China, the cotton cropping system is quite different from large-scale cotton farming in the United States and Australia, as there are many small-scale, mixed plantings of cotton, corn, soybean, peanut, and other crops planted by individual farmers (Wu et al., 2002, 2004). Non-cotton natural host crops are thought to serve as an important natural refuge for *H. armigera*, contributing to a delay in Cry1Ac-resistance evolution in *H. armigera* in the Bt cotton planting area of China (Wu, 2007; Gao et al., 2010a).

Monitoring is an integral tool for measuring potential resistance problems. Data from our previous studies of one county in China (Xiajin County, Shandong Province) indicated sustained susceptibility of *H. armigera* to Cry1Ac (Gao et al., 2010b). However, based on laboratory bioassay data, Li et al. (2007) reported a significant increase in the resistance of *H. armigera* larvae to Cry1Ac from 2002 to 2005 in the Xiajin County. Subsequent studies by Gao et al. (2009) employing the same bioassay methods used by Li et al. (2007) indicated that tolerance subsequently decreased from 2006 to 2008. For a polyphagous pest such as *H. armigera* (Wu and

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Guo, 2005), non-cotton crop hosts may act as a major factor contributing to the maintenance of *H. armigera* susceptibility to Cry1Ac. The migration of *H. armigera* over a large area may be another factor contributing to the maintenance of *H. armigera* susceptibility to Cry1Ac (Gao et al., 2010b). However, the importance of individual factors contributing to the variation in Cry1Ac susceptibility has not been studied.

Generally, wheat is the main crop host of first-generation *H. armigera* larvae in northern China, while cotton, corn, peanuts, soybeans, and vegetables may be hosts for subsequent generations (Wu and Guo, 2005). First-generation moths emerging from wheat in early summer typically migrate to cotton or corn fields. We hypothesized that there would be a significant difference in *H. armigera* susceptibility to Cry1Ac in offspring from parents that completed their development on host plants expressing this toxin versus those from parents that developed on non-Bt producing crop hosts. The objective of this work was to evaluate the contribution of non-cotton crop hosts in delaying Cry1Ac resistance in field populations of *H. armigera*.

2. Materials and methods

2.1. Sampling location

Xiajin County (36°95′ N, 116°00′ E) of Shandong Province was selected for the collection of *H. armigera* larvae. Bt cotton was introduced in this County in 1997, and by 2000, Bt cotton had almost completely replaced non-transgenic cotton cultivars. Bt corn is not currently grown in this region. Xiajin is considered a Bt cotton and corn planting system, as these two crops comprise more than 95% of the agricultural land after wheat is harvested in early-April (Table 1) (Li et al., 2007; Gao et al., 2009).

In Xiajin County, there are four generations of *H. armigera* per year. The first-generation moths emerge from wheat in early summer and migrate to Bt cotton fields, and the second-generation emerging from Bt cotton migrates to Bt cotton and to corn, where a third-generation is produced (Wu et al., 2003; Gao et al., 2010b). In the third-generation, a substantial number of *H. armigera* larvae continue to be selected on Cry1Ac Bt cotton, while some complete development on corn plants. There is a fourth-generation that develops on Bt cotton, overwinters as larvae or pupae, and emerges the following year; this generation was, however, not tested for Cry1Ac susceptibility.

Table 1
The planting history of Bt cotton and other host crop hosts of *H. armigera* during 2000–2010 in Xiajin County in northern China.

Year	Conventional cotton (%)	Bt cotton (%)	Maize (%)	Peanut (%)	Soybean (%)	Total planting area (hectare)
2000	0.00	71.41	21.98	4.16	2.44	46,400
2001	0.00	64.11	31.07	2.96	1.85	54,067
2002	0.00	69.36	25.07	3.84	1.72	50,266
2003	0.00	74.30	21.56	2.92	1.22	54,733
2004	0.00	76.65	21.23	1.77	0.35	56,533
2005	0.00	72.69	22.75	3.35	1.20	55,667
2006	0.00	68.32	26.91	3.25	1.52	55,613
2007	0.00	71.00	25.31	3.08	0.61	56,294
2008	0.00	70.98	25.34	3.09	0.59	56,328
2009	0.00	66.7	28.7	0.44	0.44	60,000
2010	0.00	69.8	24.4	0.55	0.89	60,000
2000~2010 Mean	0.00	70.48	24.94	2.67	1.17	55,081

Data on the planting history of Bt cotton and other host crops of *H. armigera* during 1998–2008 are based on Li et al. (2007) and Gao et al. (2009). All the data from 2009 to 2010 were provided by the local governments.

2.2. Insect collection and single-pair crosses

In 2008, 2009 and 2010, last instar *H. armigera* larvae or pupae were collected from wheat, Bt cotton and corn. The collection from wheat was the first-generation of the year, and the second collection was of second-generation larvae that survived on transgenic Bt cotton, while the third collection was of third-generation larvae from Bt cotton and corn. Field-collected larvae were reared on artificial diet as described in Zhou et al. (1981) in the laboratory at 27–30 °C, 70–80% RH, and a photoperiod of L14: D10. Pupae directly collected from the field or derived from rearing field-collected larvae in the laboratory, were separated by sex and transferred to 250-ml clear plastic cups for adult emergence. To develop single-pair cross isofemales lines, newly emerged males and females from each host were paired in 250-ml clear plastic cups covered with gauze fabric to provide a substrate for egg laying. Eggs were collected on a daily basis. Single-pairs and their F_1 progeny were kept under the environmental conditions described above. In 2008, a total of 118, 26, 54, and 58 isofemale lines were derived from wheat (first-generation), Bt cotton (second-generation), Bt cotton (third-generation) and corn (third-generation), respectively. In 2009, isofemale line numbers tested were 63, 16, 70 and 45, respectively, and in 2010 these numbers were 77, 14, 60, and 57, respectively.

2.3. Bioassay of F_1 larvae on Bt and non-Bt diets

Twenty-four neonates from each isofemale line were placed on either non-Bt (NBT) diet or on diet incorporated with 1.0 μg/ml of Cry1Ac. Cry1Ac toxin was obtained from Dow AgroSciences in a formulation of their product MVPII (19.1%). To avoid degradation, the toxin (liquid) was stored at −70 °C. Treated diet was poured into glass test tubes, and stored in a refrigerator (<one week before bioassays). A single neonate was placed into each test tube using a fine brush, and the test tubes were sealed with cotton plugs. Larval developmental stage was recorded after 6 days based on head capsule and body size (Table 2, see Li et al., 2004).

2.4. Data analysis

The developmental stage of each larva recorded in bioassays was first converted to a development rating based on an ordinal ranking system (Table 2). An average development rating (ADR) for each isofemale line on each of the diets in each bioassay was calculated using PROC UNIVARIATE (SAS Institute, 1998). A relative average development rating (RADR) was defined as the quotient of the larval ADR of a isofemale line on Bt diet divided by larval ADR of the same isofemale line on NBT diet (Li et al., 2004). Data on the

Table 2
Rating scale used to convert *H. armigera* developmental stage to appropriate development rating (ordinal ranking) after 6 days on diet.

Developmental stage	Development rating or ordinal ranking
Early 1st instar	1
Late 1st instar	2
Early 2nd instar	3
Mid 2nd instar	4
Late 2nd instar	5
Early 3rd instar	6
Mid 3rd instar	7
Late 3rd instar	8
Early 4th instar	9
Mid 4th instar	10
Late 4th instar	11
Early 5th instar	12