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Frequency of Bt resistance alleles in *Helicoverpa armigera* in 2007–2009 in the Henan cotton growing region of China

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ABSTRACT

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is a key insect pest of cotton in the Henan cotton growing region of China. In this region, cotton is grown on small acreages in rich agricultural landscapes, contrary to cropping systems in the United States or Australia. Under such cropping regimes, naturally occurring refuges (with non-Bt plants) may be sufficient to prevent *H. armigera* resistance development to Bt toxins. In order to gain a timely understanding of the evolution of resistance of *H. armigera* to Bt toxin after continuous cultivation of Bt cotton for c. 10 years, we assessed the frequency of alleles conferring resistance to Cry1Ac toxin in field populations of *H. armigera* sampled from Xinxiang County in Henan province during 2007–2009. Screening F_1 and F_2 generations from isofemale lines, derived from female moths trapped in the field, were used with a discriminating dose of Cry1Ac diet to estimate the frequency of resistance alleles. Totals of 625, 516 and 488 isofemale lines were screened for the F_1 generation in 2007, 2008 and 2009, respectively. Resistance gene frequency in Xinxiang fluctuated between 0.0000 and 0.0005, and it did not increase significantly from 2007 to 2009. Based on the relative average development rates (RADR) of *H. armigera* larvae in F_1 tests, no substantial increase in Cry1Ac tolerance was found in the Xinxiang region over the 3-yr period.

1. Introduction

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae), the cotton bollworm, is a serious pest in the Yellow River cotton-farming regions of China (Wu and Guo, 2005). In consideration of its importance for control of *H. armigera*, transgenic insecticidal cotton containing the Cry1Ac protein from the soil bacterium *Bacillus thuringiensis* (Bt), was first approved for planting in these regions by the Chinese government in 1997. By 2000, Bt cotton had almost completely replaced non-transgenic cotton in these regions (Wu and Guo, 2005). The planting of Bt cotton has been widely successful and is an efficient tool for controlling *H. armigera* with the reduced use of synthetic insecticides (Wu et al., 2008; Gao et al., 2010a).

The evolution of resistance in field populations of target insect pests to Bt toxin is a major threat to the continued efficacy of Bt crops (Tabashnik, 1994; Gould, 1998; Matten et al., 2008; Tabashnik and Carrière, 2009). The capacity of *H. armigera* for developing resistance to Cry1Ac has been demonstrated in several laboratory-selected strains from India, China and Australia (Kranthi et al., 2000; Liang et al., 2000; Akhurst et al., 2003), and some field populations of five Lepidopteran pests to Bt crops in South Africa, Puerto Rico, western India, southeast United States and Australia (Tabashnik et al., 2009; Carrière et al., 2010). Our previous extensive monitoring work carried out in Hebei and Shandong provinces suggested that the resistance allele frequency in field populations of *H. armigera* to Cry1Ac remains low in these regions of China (Li et al., 2004, 2007; Gao et al., 2009, 2010b). Abundant non-transgenic host crops of this pest planted closely by Bt cotton may have delayed the resistance evolution to Bt cotton in China (Wu, 2010).

Resistance monitoring program for field populations of *H. armigera* from the Henan cotton planting region has never been carried out. Henan province is located in the south of the Yellow River Region and is the major region for *H. armigera* overwintering. Winter wheat widely planted in this region, is the major host of the first generation larva (Feng et al., 2010). Bt cotton is also cultivated within highly diverse landscapes, composed of small fields of corn (*Zea mays* L.), soybean (*Glycine max* L.), cotton (*Gossypium hirsutum* L.) or peanut (*Arachis* spp.) in this province (Wu and Guo, 2005). Because of the frequent gene exchange accompanied with migration of *H. armigera* in northern China (Xu et al., 2002; Feng et al., 2009), the simultaneously evolved resistance to several pesticides

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Table 1
The planting history of Bt cotton and other host crops of the *H. armigera* during 2007–2009 in Xinxiang county of Henan province (Data provided by the local government).

Year	Bt Cotton (%)	Conventional Cotton (%)	Maize (%)	Peanut (%)	Soybean (%)	Total planting area (hectare)
1998	7.39	3.00	55.35	23.80	10.46	271, 467
1999	7.43	2.00	54.26	25.90	10.41	258, 133
2000	9.29	0.00	50.74	29.83	10.14	267, 533
2001	8.81	0.00	52.71	29.62	8.86	273, 933
2002	8.16	0.00	55.70	27.53	8.61	270, 267
2003	10.03	0.00	55.86	25.93	8.17	271, 733
2004	10.46	0.00	57.87	23.49	8.18	281, 200
2005	8.43	0.00	59.85	23.62	8.10	277, 467
2006	10.39	0.00	58.87	22.58	8.16	299, 067
2007	8.30	0.00	61.17	22.52	8.01	301, 333
2008	6.71	0.00	63.52	26.41	3.36	315, 800
2009	5.95	0.00	55.51	23.45	3.00	361, 867

has been documented for this species in the Yellow River Region (Wu and Guo, 2000). Similarly, the resistance allele frequencies in Henan populations of *H. armigera* to Cry1Ac may also reflect the average resistance level of this species to Bt cotton currently planted in wide areas of northern China. The huge number of the first generation moths produced in winter wheat facilitates the monitoring work with the F_1/F_2 screening method.

In order to gain a timely understanding of the evolution of resistance of *H. armigera* to the Bt toxin after continuous cultivation of Bt cotton c. 10 years, we estimated the frequency of alleles conferring resistance to the Cry1Ac toxin and the quantitative shifts in larval Cry1Ac tolerance of field populations of *H. armigera* from Henan (Xinxiang county) in the south of the Yellow River Region over a 3-yr period.

2. Materials and methods

2.1. Sampling field population

The field population of adult *H. armigera* was sampled in 2007–2009, with a 1000 W light-trap at an experimental station in Xinxiang county (113°48′ E, 35°09′ N), Henan province of China. Wheat is the main host crop of the 1st generation of *H. armigera*, and maize, cotton, peanut and soybean are main host crops of the subsequent generations in this region. The latter four crops are all typically planted after wheat is harvested in early summer. Bt cotton has been adopted since 1997 and has replaced non-Bt cotton completely since 2000 (Table 1).

The light-trap is made up of an upward pointing search light with a 1000 W metal halid lamp installed in a big metal funnel, which is effective to collect *H. armigera* moths flying from distance up to hundreds of meters (Feng et al., 2003). Trapped females were individually placed into 250 ml clear plastic cups that were covered with gauze as oviposition substrate. Adult females were fed with 10% honey water and were kept at 27–30 °C, 70–80% RH and L: D 14: 10. Eggs were collected on a daily basis and incubated under the same laboratory condition.

2.2. Bioassay of F_1 generation on Bt and non-Bt diets

If a field trapped female moth could produce more than 50 eggs daily, then the subsequent neonates were used for F_1 bioassays. The neonates from the same female, i.e., an isofemale line, were divided into two groups, with 24–35 neonates being individually reared on non-Bt (NBT) diet and 24–35 neonates on Cry1Ac-containing diet under the same laboratory conditions as above. After six days, the developmental stage of each individual larva was determined using head capsule and body size as indicators (Li et al., 2004, 2007, 2010). Artificial diets were prepared according to Zhou et al. (1981), and Bt toxin was added at 1.0 μg of Cry1Ac/ml of diet. Cry1Ac was provided by Dow AgroSciences in a formulation of their product MVPII (19.1%), and stored at −70 °C until incorporation into diet. Both non-Bt and Bt artificial diets were kept in a refrigerator before bioassay.

2.3. Bioassay of F_2 generation on Bt and non-Bt diets

The isofemale lines, that developed well on Bt diet during F_1 bioassay and thus were suspected carrying resistance allele (Li et al., 2007), were saved for F_2 bioassays. Specifically, the isofemale lines that performed about 80% as well on Bt diet as they did on non-Bt (NBT) diet during F_1 bioassay were saved for testing the F_2 generation. For each isofemale line that was saved, the individuals that had developed on the NBT diet were reared to adult emergence and mass sib-mated in a mating cage. The subsequent neonates, i.e., the F_2 generation, of each female line were then divided into two groups, with 24–35 neonates being individually reared on NBT diet and 24–35 neonates on Bt diet. Bioassays for F_2 generation were the same as for the F_1 generation described above. Total numbers of 30, 60 and 75 isofemale lines were successfully mass sib-mated and therefore were tested for the F_2 generation in 2007, 2008 and 2009, respectively.

2.4. Data analysis

The developmental stage of each larva recorded in the bioassays was firstly converted to a development rating (DR) basing on an ordinal ranking system (Table 2), and an average development rating (ADR) for each isofemale lines on each of the diets in each bioassays was calculated using PROC UNIVARIATE (SAS Institute, 1988). A relative average development rating (RADR), defined as

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