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The influence of pesticide applications on *Helicoverpa armigera* Hübner and sucking pests in transgenic *Bt* cotton and non-transgenic cotton in China

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Abstract

Effects of pesticide applications, based on an IPM program on cotton bollworm, *Helicoverpa armigera* Hübner, cotton mirids and cotton leafhoppers, were evaluated in transgenic *Bt*-cotton and non-transgenic cotton agroecosystems between 1999 and 2001 in China. Differences in pest populations between cotton varieties were also compared.

In 1999 and 2000, bollworm populations on non-transgenic cotton were larger than those on transgenic *Bt*-cotton. In *Bt*-cotton fields, the numbers of fourth-generation bollworms were greater than those of in the second and the third generations over all 3 years of study. Leafhopper populations on *Bt*-cotton were consistently larger than those on non-transgenic cotton during the 3 years of study. Although the use of transgenic *Bt*-cotton decreased the need for insecticide applications against cotton bollworm, this relaxation from pesticide applications could cause increased populations of sucking insects, which could require additional insecticide applications.

Keywords: Transgenic *Bt*-cotton; Pesticides; Bollworm (*Helicoverpa armigera*); Mirids; Leafhoppers

1. Introduction

Transgenic cotton expressing δ-endotoxin genes such as HD-1 (Cry1A(b)) and HD-73 (CryIA(c)), from the bacterium *Bacillus thuringiensis* (Perlak et al., 1990) has received considerable attention for its potential to control cotton bollworm, *Helicoverpa armigera* Hübner, an important pest of cotton because it has developed resistance to most of the insecticide groups used commonly on cotton, such as organochlorines, organophosphates, carbamates, pyrethroits and cyclodienes (Forrester et al., 1993). Transgenic cotton also offers the potential to decrease the use of broad-spectrum chemical insecticides to control lepidopterous pests (Fitt, 1994; Joanne, 1994) and promote relative safety for non-target organisms (Huang et al., 1999; Meeusen and Warren, 1989).

An increasing number of studies have focused on the assessment of ecological risks associated with *Bt*-cotton, with an emphasis on its effects on both target pests and their natural enemies (Fitt et al., 1994; Frutos et al., 1999; Huang et al., 1999; Joanne, 1994; Smith, 1997; Van Tol and Lentz, 1998). In 1998, transgenic *Bt*-cotton was introduced into China to manage insecticide resistant strains of the cotton bollworm. Huang et al. (2002) showed that resistance of *Bt*-cotton to *H. armigera* decreased gradually from July to September. In most cases, *Bt*-cotton has been planted without any

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Table 1
Pesticide applications in the pesticide-treated non-transgenic and *Bt*-cotton plots during the 3 years of study (Insecticides applied on the same date were sprayed as mixtures)

Pesticides	Target pests	Action threshold	Application rate (a.i.) (per ha)	Non-transgenic cotton			*Bt*-cotton		
				1999	2000	2001	1999	2000	2001
Dicofol	Cotton mites	70 adults/plant	113 g				25-Jun		
Phosalone	Cotton bollworms	100 eggs/100 plant	505 g	22-Jul		20-Jul			
Monocrotophos	Cotton mirids	10 adults/100 plants	150 g				10-Jul		10-Jul
Cypermethrin	Cotton aphids	2000 individuals/100 plants	46 g	22-Jul	18-Jul	20-Jul	22-Jul		
Omethoate	Cotton aphids	2000 individuals/100 plants	180 g	9-Aug		8-Aug	20-Aug		8-Aug
Carbaryl	Cotton leafhoppers	200 individuals/100 plants	650 g			8-Aug	20-Aug	20-Aug	8-Aug

risk assessment studies dealing with any adverse effects on agroecosystems. Therefore, it is very important to investigate any potential ecological risks from the transgenic *Bt*-cotton to justify its long-term use in China.

Most studies dealing with the effects of *Bt*-varieties have compared *Bt*-plants with their isogenic or parental lines. Our experiments focused on a comparison of two cotton varieties (Bt, Deltapine and Chun Aizao) which have similar agronomic characteristics and are preferred by farmers in the Chinese Cotton Belt (Huanghe River Valley). This type of comparison is particularly important in evaluating the consequences of introducing a new transgenic variety into an area if it should replace a local variety. *Bt*-cotton is used against insecticide-resistant strains of cotton bollworms. Our objective was to evaluate the effects of pesticide applications, based on an IPM program for cotton bollworm and two sucking pests, mirids and leafhoppers, on transgenic *Bt*-cotton and local non-transgenic cotton varieties. Differences in pest populations between the two cotton varieties were also compared.

2. Materials and methods

2.1. Description of field experiments

The field experiment was carried out in Fugou County, Henan province, China (34N, 115E), where wheat and cotton are the main crops, and are often intercropped. Wheat was sown in October and harvested in June of the following year. Cotton was sown into the wheat field in May and harvested in October of the same year. The experimental field had a medium-textured silty loam soil, each year it was fertilized with 30 kg urea, 120 kg calcium superphosphate per ha on 20 June and 50 kg urea, 180 kg calcium superhposphate per ha on 25 July. Transgenic *Bt*-cotton and non-transgenic cotton seeds were sown at similar rates to provide 60,000 plants/ha.

Transgenic *Bt*-cotton (Deltapine NuCOTN 33B) containing the Bollgard gene expressing Cry1 A(c) (Monsanto, ST. Louis, MO) and the non-transgenic cotton (Chun Aizao) were planted in 1999–2001. They germinate early with a life span of about 130 days; are of medium height and have a deep green leaf color. The experiment was based on a randomized complete block design, involving four treatments each with three replicate plots. Each replicate plot was 0.4 ha and the same plots were used throughout the experiments. The treatments included: (i) non-transgenic cotton without pesticide applications, (ii) non-transgenic cotton with pesticide applications, (iii) *Bt*-cotton without pesticide applications and (iv) *Bt*-cotton with pesticide applications. For both cotton varieties, insecticides were selected and applied according to an IPM program, which was designed to control the whole pest complex in conventional (non-transgenic) cotton agroecosystems in China (Chen et al., 1990). The program was based on applications of insecticides whenever the population of pests exceeded action thresholds. The same pesticides were used in all years. Table 1 summarizes the types of pesticides, the action thresholds and the dosages used for the 3 years in the non-transgenic and the Bt plots.

2.2. Pest and predator sampling

Pests that were sampled included cotton bollworm, *Helicoverpa armigera* Hübner, mirids (*Adelphocoris lineolatus* (Goeze), *A. fasciaticollis* (Reuter) and *A. suturalis* (Jakovlev)) and leafhoppers (*Empoasca biguttula* (Shiraki)). Five 1-m^2 sampling sites, each consisting of six cotton plants, were selected randomly in each plot. Arthropods were counted visually on all plants at each site once every five days from 15 May to 10 September in all years. All the control decisions were made based on action threshold levels (number of pests per 100 plants).

2.3. Data analyses

Data were analyzed by one-way ANOVA using SPSS (SPSS Institute). Means were separated by Duncan's Multiple Range test at the five-percent probability level.

Effects of years, pesticides and varieties were analyzed using GLM procedure.

3. Results

3.1. Pest populations

In 1999, cotton bollworm populations differed significantly between treatments ($F=24.21$; df $=3$, 8; $P<0.001$) (Figs. 1 and 2). The pesticide applications to non-transgenic and *Bt*-cottons caused significant decreases in cotton bollworm populations compared with those in non-transgenic plots without any pesticide applications and *Bt* plots without pesticide applications, respectively. Cotton bollworm populations on non-transgenic cotton with no pesticide treatments were significantly larger than those on *Bt*-cotton with no pesticide applications ($F=14.132$; df $=1$, 4; $P=0.02$). Fourth-generation cotton bollworm populations were significantly larger than those of the second and the third generations on *Bt*-cotton ($F=14.893$; df $=2$, 6; $P=0.002$) (Fig. 2).

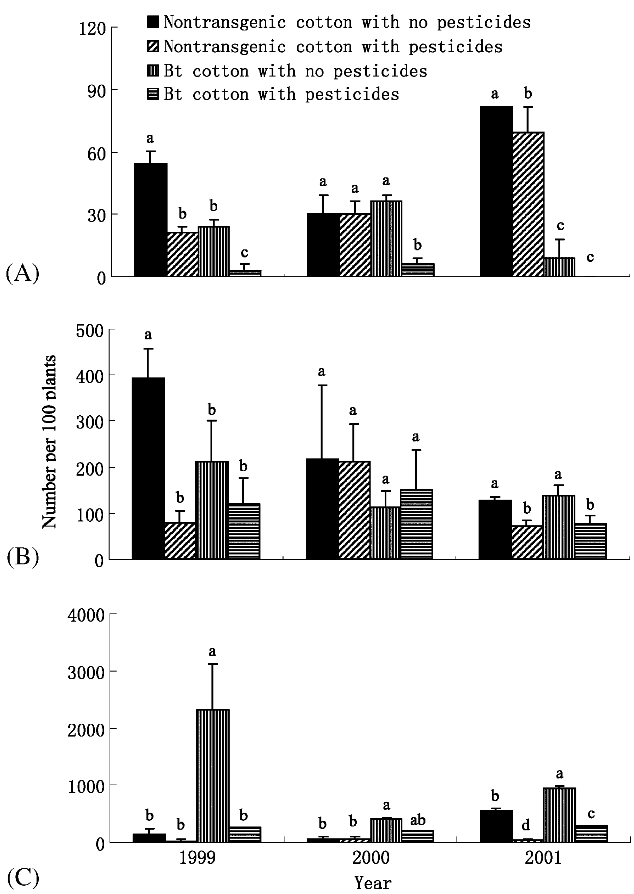

Fig. 1. Populations (Mean ± SEM) of cotton bollworm larva, mirids and leafhoppers in the experimental plots in 1999, 2000 and 2001 (in a year, bars indicated by different letters are significantly different at $p<0.05$). (A) cotton bollworm; (B) mirids; (C) leafhoppers.

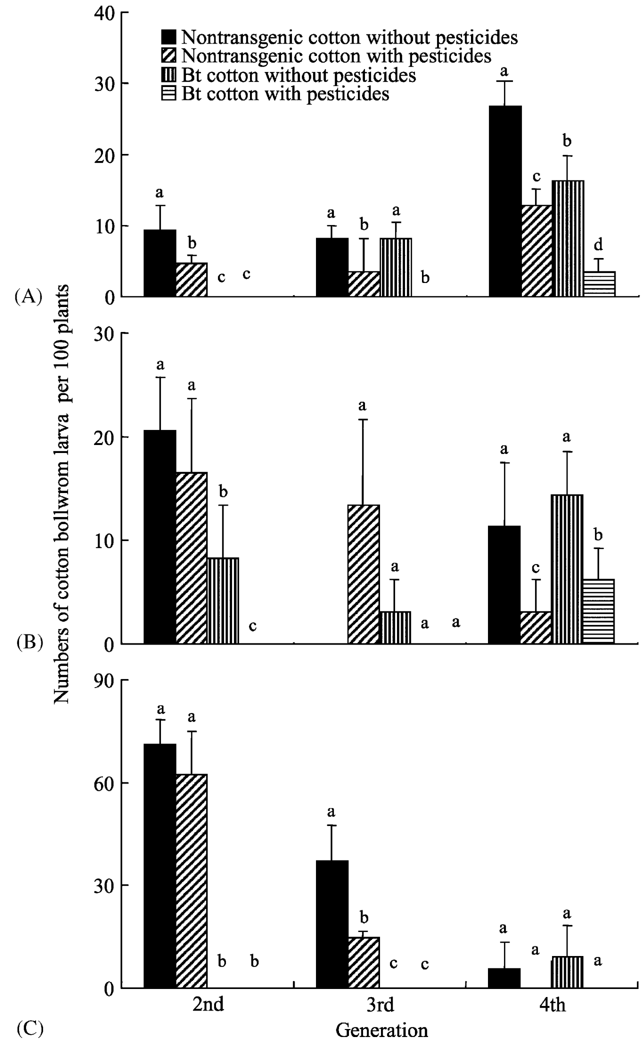

Fig. 2. Populations (Mean ± SEM) of cotton bollworm larva in the 2nd, 3rd and 4th generations in the four cotton agroecosystems in 1999, 2000 and 2001. (A) 1999; (B) 2000; (C) 2001.

In 2000, pesticides decreased the populations of bollworms significantly only on the *Bt*-cotton ($F=34.031$; df $=1$,4; $P=0.01$) (Fig. 1). No significant differences occurred between varieties. On *Bt*-cotton, bollworms of the forth generation were more numerous ($F=9.324$; df $=2$, 6; $P=0.014$) than those of the second and the third generations; however, on non-transgenic cotton, bollworms of the second generation were more numerous ($F=10.889$; df $=1$, 4; $P=0.030$) than those of the fourth generation (Fig. 2).

In 2001, pesticides decreased bollworm populations significantly on non-transgenic cotton ($F=76.00$; df $=1$, 4; $P<0.001$). No cotton bollworms were detected on *Bt*-cotton treated with pesticides.

In all years, numbers of bollworms of the second generation on non-transgenic cotton with no pesticides were higher than those on *Bt*-cotton with no pesticides ($F=30.206$; df $=1$,4; $P=0.005$ in 1999; $F=8.467$; df $=1$, 4; $P=0.042$ in 2000; $F=166.705$, df=1,4; $P<0.001$ in 2001).

In 1999, pesticide applications reduced mirid populations significantly only on non-transgenic cotton ($F=20.297$; df=1, 4; $P=0.011$) and mirid numbers were greater on non-transgenic cotton with no pesticide applications than on *Bt*-cotton with no pesticide applications ($F=11.778$; df=1, 4; $P=0.027$) (Fig. 1). Pesticides depressed mirid populations significantly on *Bt*-cotton ($F=33.047$; df=1, 4; $P=0.001$) and on non-transgenic cotton ($F=18.206$; df=1, 4; $P=0.013$) in 2001.

Overall leafhopper populations on *Bt*-cotton were significantly greater than those on non-transgenic cotton in 1999 ($F=34.908$; df=3, 8; $P<0.001$), 2000 ($F=21.912$; df=3, 8; $P=0.009$) and 2001 ($F=54.876$; df=3, 8; $P<0.001$). Pesticides decreased leafhopper populations significantly on non-transgenic cotton ($F=401.929$; df=1, 4; $P<0.001$) and on *Bt*-cotton ($F=263.087$; df=1, 4; $P<0.001$) in 2001.

3.2. Multi-factor effects

A summary of the statistical analyses on the effects of years, pesticide treatments and cotton varieties on mean populations of pests are summarized in Table 2.

There were significant differences in leafhopper populations among the 3 years ($P<0.001$). The pesticide treatments reduced populations of leafhoppers ($P<0.001$) and mirids ($P<0.01$) significantly; varieties influenced the bollworm and leafhopper ($P<0.001$) populations significantly. There were significant interactions between years and pesticide treatments (Year∗Pesticide) for leafhopper ($P<0.001$) and mirid ($P<0.05$) populations. Interactions between years and varieties (Year∗Variety) and interactions among all three factors (Year∗Pesticide∗Variety) were significant for leafhopper ($P<0.001$) populations.

Table 2
F-test on effects of years, pesticide-treated and cotton varieties on the abundance of pests

Source of variation	df	*F*-values		
		Bollworms	Mirids	Leafhoppers
Year	2	0.96	2.43	13.29***
Pesticide	1	3.79	7.73**	25.63***
Variety	1	11.75**	2.44	32.94***
Year∗Pesticide	2	0.18	3.99*	18.10***
Year∗Variety	2	3.12	0.6	14.12***
Pesticide∗Variety	1	0.06	0.85	8.36**
Year∗Pesticide∗Variety	2	0.76	2.62	16.71***

*$P<0.01$,
**$P<0.001$,
***$P<0.0001$.

4. Discussion

Although, field experiments have demonstrated that *Bt*-cotton can be effective against certain lepidopteran pests (Bacheler and Mott, 1996; Bacheler et al., 1998; Davis et al., 1995; Layton, 1998; Luttrell et al., 1995; Watson, 1995), it may not be potent enough to control cotton bollworm adequately. It has been reported that *Bt*-cotton has sometimes failed to provide season-long control of *H. armigera* in Australia (Hilder and Boulter, 1999). It has also been reported that *H. armigera* is more tolerant to transgenic *Bt*-plants than other target bollworms such as *H. punctigera* (Fitt et al., 1994). The results of the present experiments indicate that *Bt*-cotton provided a degree of protection against cotton bollworm and kept populations under the action threshold in the *Bt*-cotton plots. In the non-transgenic cotton plots, pesticides were required to reduce populations of bollworms below the action threshold for pesticide use.

In China, the first generation of *H. armigera* occurs in wheat fields; the second generation usually damages the cotton tips, the third generation feeds on squares, and the fourth generation causes damage to cotton bolls, because few flower buds and cotton tips have been removed at this time. During the second generation of *H. armigera*, cotton plants have a high capacity for self-compensation; therefore the action threshold for chemical control can be higher. However, the self-compensation capacity of cotton is relatively low during the third and fourth generations of cotton bollworms, and individuals can cause economic damage directly to cotton. Therefore, the third and fourth generations are key stages for economic control of bollworms. (Cotton Insect Research Group, 1995). Populations of the fourth generation of bollworms were consistently greater than those of the second and third generations on *Bt*-cotton in all 3 years. This could be because the toxins expressed in *Bt*-cotton plants decreased gradually with the seasonal senescence of cotton plants (Fitt et al., 1994; Zhao et al., 1998). Olsen and Daly (2000) reported similar results from Fitt et al. (1994) and Fitt (1998) that efficacy of Bt toxin declines after flowering and survival of larvae increases as the plant matures.

The insecticide applications, which were based on the IPM program, provided a significant degree of bollworm control on non-transgenic cotton. Although, insecticides were not directly applied against bollworms, because their populations did not exceed the action threshold on *Bt*-cotton, and were targeted against other pests, they still caused significant decreases in cotton bollworm populations. Many of the non-target pests were not susceptible to any of the current Cry 1A(c) *Bt*-cotton strains, so *Bt*-cotton may still need to be sprayed with pesticides against some other pests, such as lepidopteran pests (Fitt et al., 1994).

The lack of significant differences in cotton bollworm populations, between non-transgenic cotton with no pesticide applications and *Bt*-cotton with no pesticide applications as well as between the other pest populations in plots in 2000 might be due to the fact that heavy and long-lasting rains depressed populations of bollworms (Chen et al., 2003) as well as other pests in all treatment plots. The total rainfall from June to September in 2000 was about 160% and 187% of that in 1999 and 2001, respectively (Table 3).

Mirid populations on *Bt*-cotton exceeded the pesticide action threshold (10 adults/100 plants) in 1999 and 2001 and received insecticide applications, which decreased populations significantly. However, mirid populations on non-transgenic cotton did not need any insecticide applications in any of the 3 years. Significant decreases in mirid populations on non-transgenic plants treated with insecticides could have resulted from insecticide applications targeting other pests.

Consistently larger leafhopper populations on *Bt*-cotton in all 3 years could indicate that this cotton variety might be more suitable for the development of leafhoppers than non-transgenic cotton and might increase the need for insecticide applications. It has been reported that whitemarked leafhopper (S*panagonicus albofasciatus* Reuter) populations on *Bt*-cotton were larger than those on non-transgenic cotton (Wilson et al., 1992). In our experiments, the populations of leafhoppers on non-transgenic cotton exceeded the pesticide action threshold (200 individuals/100 plants) only in 2001. However, leafhoppers on *Bt*-cotton needed insecticide applications in all 3 years.

An expectation of farmers using *Bt*-cotton is to be able to decrease pesticide usage and cost. Over the 3 years of our experiment a total of eight insecticide sprays (only two of them against cotton bollworms) were needed against the pest complex on the local non-transgenic variety in all 3 years, while nine insecticide applications were required against sucking pests only on *Bt*-cotton.

In conclusion, our data indicated that both pesticide applications and growing the *Bt*-cotton variety could provide protection against cotton bollworms. In general, pesticide applications could decrease populations of sucking insects effectively. However, consistent increases in leafhopper populations as well as later generations of bollworms on *Bt*-cotton may need more attention. A lack of differences in pesticide usage between treatments may require cost:benefit analyses for *Bt*-cotton, particularly in relation to its long-term use in China in areas like Henan, where the bollworm pressure was very light in these years.

Table 3
Cumulative rainfall (mm) in a month from May to October in 1999, 2000 and 2001

Year	Month					
	May	June	July	August	September	October
1999	100.5	36.3	332.1	48.1	169.5	70.9
2000	43.2	193.5	381	106.8	258.5	63.9
2001	1.2	66.3	426.8	8.3	1.9	36.4

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