# Influence of Pesticide Applications on Pest and Predatory Arthropods Associated with Transgenic *Bt* Cotton and Nontransgenic Cotton Plants

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The effects of pesticide applications on pests (aphids and acarid mites) and predators (ladybeetles and spiders) were investigated in transgenic *Bt* cotton and nontransgenic cotton agroecosystems in 1999, 2000 and 2001. Transgenic cotton did not cause changes in populations of acarids and did not reduce numbers of predators considerably; its effects on aphids were inconsistent. Although insecticides were not applied against the main pest – cotton bollworm – on transgenic cotton, the total number of insecticide applications in 3 years was no less than the total applied on nontransgenic cotton, because additional applications were required against sucking pests on transgenic *Bt* cotton. Pesticide applications decreased numbers of aphids, acarids and predatory spiders significantly on both transgenic and nontransgenic cottons. The results suggest that the use of *Bt* cotton should be evaluated carefully in China.

KEY WORDS: Transgenic *Bt* cotton; pesticides; arthropods; pests; predators; non-target effect.

## INTRODUCTION

Control of cotton bollworm (*Helicoverpa armigera*) remains a major problem in cotton production. Transgenic cotton expressing $\beta$-endotoxin genes such as HD-1 (Cry1Ab) and HD-73 (Cry1Ac) from the bacterium *Bt* (15) is a promising new means of managing the bollworm, which has developed resistance to most of the insecticide groups such as organochlorines, organophosphates, carbamates, pyrethroids and cyclodienes (8). It also offers a potential to reduce the use of broad-spectrum chemical insecticides for controlling lepidopterous pests (6,13), with fewer effects on non-target organisms (12,14). In 1998, transgenic *Bt* cotton became available in China to control insecticide-resistant strains of the bollworm, for which farmers had previously relied heavily on insecticides that were environmentally disruptive and costly (6,11).

Studies of the effects of transgenic *Bt* cotton on target and non-target pests as well as their natural enemies have been done to assess various aspects of ecological risks (7,9,12,13,17,19). The multiple–planting pattern of cotton in China is different from the single large area planting done in America and Australia. Wheat (*Triticum aestivum* L.) is planted in winter and harvested in summer in the cotton belt in China. Many natural enemies overwinter within wheat fields and most of these arthropod predators are also

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natural enemies of cotton pests such as aphids (22), which migrate into cotton fields in the spring. Therefore, it is important to search for any ecological risks associated with the use of transgenic *Bt* cotton that could affect this establishment in China.

The objectives of this investigation were to evaluate the effects of pesticides on the major arthropod pests and their predators on transgenic *Bt* cotton and local nontransgenic cotton varieties, and to compare possible differences between varieties in terms of pest and predator populations.

## MATERIALS AND METHODS

**Description of field experiments** The experiments were conducted in Fugou County, Henan province, China (34N, 115E), where wheat and cotton are the main crops that are often intercropped. Wheat was sown in October and harvested in June of the following year. Cotton was transplanted into the wheat field in May and harvested in October of the same year. The experimental field was on a medium-textured silty loam soil, fertilized each year with 30 kg urea and 120 kg calcium superphosphate per ha on 20 June plus 50 kg urea and 180 kg calcium superphosphate per ha on 25 July.

Transgenic *Bt* cotton (Deltapine NuCOTN 33B) containing the Bollgard gene expressing Cry1Ac (Monsanto, St. Louis, MO, USA) and local nontransgenic cotton var. Chun Aizao, were used in this study because both varieties are commonly planted in the cotton belt (Huanghe River area) of China and they have similar characteristics. They are precocious, with a life span of about 130 days, and are of medium height with deep green leaf color. Both varieties were planted at similar seeding rates in 1999, 2000 and 2001. The experiment was laid out in a completely randomized block design involving four treatments with three replicates. Each replicate plot was 0.4 ha. The treatments included: (i) nontransgenic cotton without pesticide applications; (ii) nontransgenic cotton with pesticide applications; (iii) *Bt* cotton without pesticide applications; and (iv) *Bt* cotton with pesticide applications. Insecticides were selected and applied according to an IPM program that is designed to control the pest complex in conventional (nontransgenic) cotton agroecosystems in China (3). The program was based on applications of insecticides whenever the pest populations exceeded the action thresholds. The same pesticides were used in all 3 years. Table 1 summarizes the types of pesticides, action thresholds and dosages applied in nontransgenic and *Bt* plots.

**Pest and predator sampling** The main pests sampled included cotton aphids (*Aphis gossypii* Glover) and mites (*Tetranychus cinnabarinus* and *Tetranychus truncatus*); the major predators sampled were ladybeetles (*Propylaea japonica* (Thunberg)) and spiders (*Erigone* spp. and *Thomisus* spp.). In each plot, mites and predators were sampled in five randomly selected 1-$m^2$ sites, each consisting of six cotton plants, and counted visually on the plants once every 5 days from 15 May to 10 September. Aphids were sampled on five randomly selected plants in each plot and counted on plants visually on three leaves taken from three different positions within the plant canopy as described by Hardee *et al.* (10). Position 1 was the fourth fully expanded leaf from the terminal, position 2 was the first main stem green leaf about one-third the distance from the terminal, and position 3 was the first main stem green leaf above the first fruiting branch.

**Data analyses** Data were analyzed by one-way ANOVA using SPSS (SPSS Institute, Chicago, IL, USA). Means were separated by Duncan's Multiple Range Test at the 0.05 probability level. Effects of years, pesticides and varieties were analyzed using GLM procedure.

## RESULTS

**Pest populations** In 1999, aphid populations differed significantly between treatments (Table 2, Fig. 1) ($F$=11.341, df=3, $P$<0.001) and were depressed significantly by pesticide applications on nontransgenic cotton ($F$=17.884, df=3, $P$=0.013) and on *Bt* cotton ($F$=45.378, df =3, $P$=0.003). Aphids that appeared before 15 July were termed seeding aphids; those that appeared after 15 July were termed summer aphids. Seeding aphids on *Bt* cotton with no pesticides were more numerous than on nontransgenic cotton with no pesticides ($F$=8.726, df=3, $P$=0.032), whereas summer aphids on nontransgenic cotton without pesticides were more numerous than those on *Bt* cotton with no pesticides ($F$=14.04, df=3, $P$=0.027) (Fig. 1).

Aphid populations did not differ significantly between treatments in 2000 ($F$=0.192, df=3, $P$=0.900), but did differ significantly in 2001 ($F$=7.741, df=3, $P$=0.039). Pesticides used on nontransgenic cotton ($F$=122.104, df=3, $P$<0.001) and *Bt* cotton ($F$=17.449, df=3, $P$=0.014) reduced aphid numbers significantly. Heavy rains in July (Table 3) led to decreased density of seeding aphids (Fig. 1). Populations of summer aphids were up to 100% higher on nontransgenic cotton with no pesticides than on *Bt* cotton with no pesticides ($F$=149.523, df=3, $P$<0.001).

There were significant differences in acarid mite populations between treatments in 1999 ($F$=7.758, df=3, $P$=0.002) (Table 2). Pesticide applications reduced the acarid mite populations on nontransgenic cotton ($F$=12.809, df=3, $P$=0.023) and on *Bt* cotton ($F$=14.022, df=3, $P$=0.020) significantly, compared with no pesticide treatments. Acarid populations did not differ between nontransgenic cotton and *Bt* cotton when neither received pesticide applications ($F$=1.551, df=3, $P$=0.248). No significant differences occurred with respect to acarid populations in treatment plots in 2000 and 2001.

**Predator populations** Pesticide treatments did not influence ladybeetle populations significantly on nontransgenic cotton ($F$=3.781, df=3, $P$=0.124) or *Bt* cotton ($F$=3.062, df=3, $P$=0.155) in any year. Numbers of ladybeetles on nontransgenic cotton with no pesticides were significantly greater ($F$=23.592, df=3, $P$=0.011) than those on *Bt* cotton with no pesticides in 2001 (Table 4).

Spider populations on *Bt* cotton with no pesticide treatment were greater than those on nontransgenic cotton with no pesticides in 1999 ($F$=14.019, df=3, $P$=0.020) and 2000 ($F$=12.098, df=3, $P$=0.020). Spider populations were depressed significantly by pesticides on nontransgenic cotton ($F$=66.003, df=3, $P$<0.001) and on *Bt* cotton ($F$=24.673, df=3, $P$=0.011) in 1999 (Table 4) and in 2001: on nontransgenic cotton ($F$=53.890, df=3, $P$=0.002) and on *Bt* cotton ($F$=81.126, df=3, $P$<0.001).

**Multi-factor effects** A summary is given of statistical analyses on the effects of years, pesticide treatments and cotton varieties on the mean abundance of pests (Table 5) and natural enemies (Table 6).

The differences in aphid and acarid mite populations among the 3 years were significant ($P$<0.001). Pesticide treatments influenced populations of both aphids ($P$<0.001) and acarid mites ($P$<0.001); varieties influenced only the aphid populations significantly ($P$<0.001). There were significant ($P$<0.001) interactions between years and pesticide treatments (Year*Pesticide) for aphid and acarid mite populations. Interactions between years and varieties (Year*Variety) were significant ($P$<0.05) only for aphid populations. No significant interaction among three factors (Year*Pesticide*Variety) occurred.

TABLE 1. Pesticide applications in nontransgenic 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 | Rate of application (a.i., g $ha^{-1}$) | Year | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Nontransgenic cotton | | | *Bt*-cotton | | |
| | | | | 1999 | 2000 | 2001 | 1999 | 2000 | 2001 |
| Dicofol | Cotton mites | 70 adults/plant | 113 | | | | 25 Jun | | |
| Phosalone | Cotton bollworms | 100 eggs/100 plants | 505 | 22 July | | 20 July | | | |
| Monocrotophos | Cotton mirids | 10 adults/100 plants | 150 | | | | 10 July | | 10 July |
| Cypermethrin | Cotton aphids | 2000 individuals/100 plants | 46 | 22 July | 18 July | 20 July | 22 July | | |
| Omethoate | Cotton aphids | 2000 individuals/100 plants | 180 | 9 Aug. | | 8 Aug. | 20 Aug. | | 8 Aug. |
| Carbaryl | Cotton leafhoppers | 200 individuals/100 plants | 650 | | | 8 Aug. | 20 Aug. | 20 Aug. | 8 Aug. |

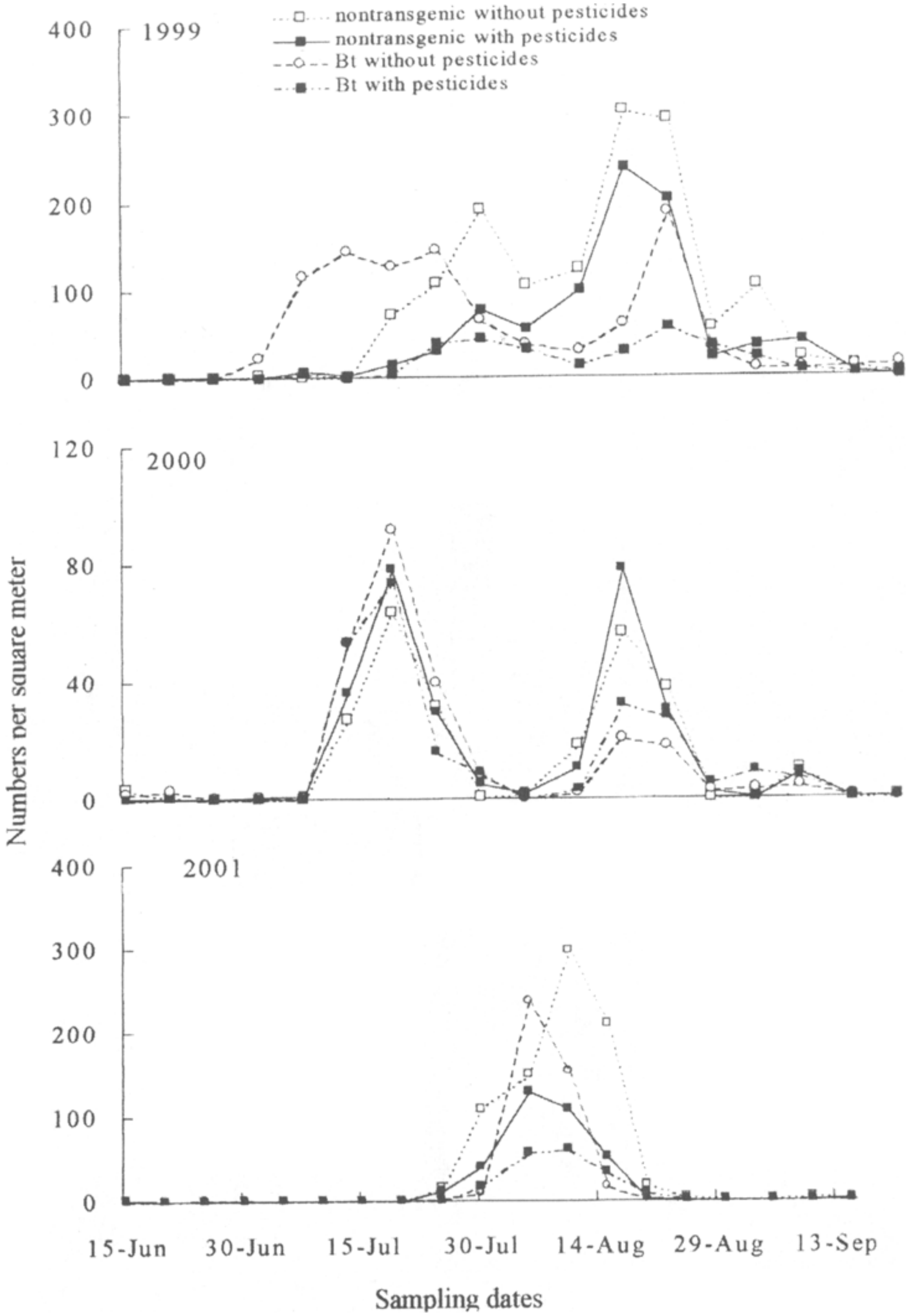


Fig. 1. Seasonal dynamics of cotton aphid populations in 1999, 2000 and 2001.

TABLE 2. Mean seasonal abundance (mean±S.E.M.) of the main arthropod pests per square meter in experimental plots in 3 years

| Year | Pest | Nontransgenic cotton | | *Bt* cotton | |
|---|---|---|---|---|---|
| | | no pesticides | with pesticides | no pesticides | with pesticides |
| 1999 | Aphids | 76.67±17.49a[z] | 46.03±7.46 b | 55.67±26.33 ab | 16.85±5.80 c |
| | Acarids | 140.00±32.47 a | 54.47±17.95 bc | 97.07±29.57 ab | 25.00±4.51 c |
| 2000 | Aphids | 13.63±2.05 a | 15.60±3.65 a | 13.73±2.18 a | 13.08±0.17 a |
| | Acarids | 2.26±0.45 a | 2.78±1.64 a | 6.17±2.83 a | 1.31±0.77 a |
| 2001 | Aphids | 40.57±5.11 a | 17.42±0.55 b | 21.19±5.68 b | 9.08±0.41 c |
| | Acarids | 0.33±0.13 a | 0.11±0.07 a | 0.67±0.07 a | 0.9±0.09 a |

[z]Within rows, values followed by a common letter do not differ significantly at $P<0.05$.

TABLE 3. Average daily rainfall (mm) from May to October in 3 years

| Year | Month | | | | | |
|---|---|---|---|---|---|---|
| | May | June | July | Aug. | Sept. | Oct. |
| 1999 | 100.5 | 36.3 | 332.1 | 48.1 | 169.5 | 70.9 |
| 2000 | 43.2 | 193.5 | 381.0 | 106.8 | 258.5 | 63.9 |
| 2001 | 1.2 | 66.3 | 426.8 | 8.3 | 1.9 | 36.4 |

TABLE 4. Mean seasonal abundance (mean±S.E.M.) of predators per square meter in experimental

| Year | Predator | Nontransgenic cotton | | *Bt* cotton | |
|---|---|---|---|---|---|
| | | no pesticides | with pesticides | no pesticides | with pesticides |
| 1999 | Ladybeetles | 0.73±0.35 a[z] | 1.04±0.46 a | 0.96±0.46 a | 0.58±0.20 a |
| | Spiders | 4.66±0.81 b | 1.58±0.26 c | 6.01±1.37 a | 1.84±0.40 c |
| 2000 | Ladybeetles | 0.89±0.35 a | 0.68±0.24 a | 0.83±0.09 a | 1.17±0.39 a |
| | Spiders | 0.77±0.65 bc | 0.46±0.29 c | 1.37±0.26 a | 1.12±0.28 ab |
| 2001 | Ladybeetles | 0.35±0.04 a | 0.44±0.07 a | 0.23±0.01 b | 0.17±0.01 b |
| | Spiders | 1.25±0.60 a | 0.10±0.02 b | 1.49±0.23 a | 0.27±0.00 b |

[z]Within rows, values followed by a common letter do not differ significantly at $P<0.05$.

TABLE 5. $F$-test on effects of year, pesticide treatment and cotton variety on the mean seasonal abundance of pests

| | df | Aphids | Acarids |
|---|---|---|---|
| Years | 2 | 45.32*** | 46.95*** |
| Pesticides | 1 | 7.08** | 8.21** |
| Varieties | 1 | 12.80** | 1.7 |
| Years*Pesticides | 2 | 13.57*** | 13.01*** |
| Years*Varieties | 2 | 5.09* | 2.82 |
| Pesticides*Varieties | 1 | 3.3 | 0.16 |
| Years*Pesticides*Varieties | 2 | 1.83 | 0.05 |

Significant difference: ***$P<0.001$; **$P<0.01$; *$P<0.05$.

Spider populations differed significantly ($P<0.001$) among years. Pesticides ($P<0.001$) and varieties ($P<0.01$) influenced spider populations significantly. The interaction between years and pesticide treatments was significant ($P<0.001$) for spider populations, and that between pesticide treatments and variety was significant ($P<0.01$) for ladybeetle populations.

TABLE 6. $F$-test on effects of year, pesticide treatment and cotton variety on the mean seasonal abundance of predators

| | df | Ladybeetles | Spiders |
|---|---|---|---|
| Years | 2 | 1.19 | 101.53*** |
| Pesticides | 1 | 0.07 | 57.16*** |
| Varieties | 1 | 0.11 | 7.53** |
| Years*Pesticides | 2 | 0.02 | 68.35** |
| Years*Varieties | 2 | 1.84 | 0.66 |
| Pesticides*Varieties | 1 | 8.07** | 1.96 |
| Years*Pesticides*Varieties | 2 | 0.5 | 0.94 |

Significant difference: ***$P<0.001$; **$P<0.01$; *$P<0.05$.

## DISCUSSION

In this study, untransformed cotton var. 'Deltapine' was not used to compare with the transgenic *Bt* Deltapine cotton because it is not used in the cotton belt of China (Huanghe River Area). Instead, a local nontransgenic variety, 'Chun Aizao', which is not isogenic or a parental line of Deltapine, was used in comparison with the *Bt* Deltapine because farmers commonly use it. *Bt* Deltapine has been introduced into the cotton belt because it shares similar characteristics with Chun Aizao and provides control of cotton bollworm. This type of comparison is particularly important in evaluating the consequences of introducing into an area a transgenic variety with a chance of replacing a local variety. One of the expected consequences of introducing *Bt* cotton is a reduction in the number of insecticide sprays needed because it provides resistance to certain lepidopteran pests (cotton bollworm in our case) (1,12). This might result in increases in other pest populations that would be suppressed by insecticide applications primarily targeting lepidopteran pests. Therefore, insecticide applications might still be needed to control these pests in transgenic cottons. In addition, introducing a transgenic variety may lead to a variation in insecticide application regimes based on an IPM program, because it may better attract some pests and/or enhance growth of pest populations in response to a difference in variety. Hence, pest populations may reach action thresholds at different times or acquire additional insecticide applications. It is also important to evaluate ecological effects associated with this type of possible variations in pesticide application regimes, which might affect pest and predatory arthropod populations regardless of *Bt* toxin. In our study, despite the fact that the same action thresholds were used, different insecticide regimes were applied against the pest complex on transgenic and nontransgenic cottons, because insecticide sprays were not required against cotton bollworms on transgenic *Bt* cotton and populations of other pests grew differently and reached action thresholds at different times. A total of eight insecticide sprays (two of them against the bollworm) were applied against the pest complex on the local nontransgenic variety in 3 years. However, nine insecticide applications were needed only against sucking pests on *Bt* cotton. Despite different application regimes, similar effects on aphids, acarids and predatory arthropods were observed in response to sprays on *Bt* cotton and nontransgenic cotton.

Lack of significant differences in arthropod populations between treatment plots in 2000 might be due to the fact that heavy and extended rainfall depressed pest populations

(2) in all treatment plots. The total rainfall from June to September in 2000 was approximately 160% and 187% of that in 1999 and 2001, respectively (Table 3).

Aphid populations were significantly affected by pesticides on both nontransgenic and *Bt* cottons. No insecticide application was needed against seeding aphids on *Bt* cotton in 2001. Most probably, insecticide application against other pests kept aphid densities under the action threshold. In 1999, with no pesticides, seeding aphids were more numerous on *Bt* cotton than on nontransgenic cotton; however, summer aphid populations were greater on nontransgenic cotton than on *Bt* cotton. The mechanism behind this difference is not clear and remains to be investigated.

Acarid mites did not show different responses to varieties. Although an insecticide was not applied specifically against mites on nontransgenic cotton in 1999, when mite populations were relatively high, applications against other pests decreased the densities of acarid mites significantly.

Insecticides did not reduce ladybeetle populations significantly. This could indicate that *(a)* the populations were in equilibrium even when affected by pesticides; *(b)* ladybeetles could survive the insecticides; or *(c)* ladybeetles in neighboring plots could emigrate in a short time to the plots where pesticides are sprayed. There were no significant differences in ladybeetle populations on *Bt* cotton and on nontransgenic cotton in 1999 or 2000, but in 2001 the populations were greater on nontransgenic cotton than on *Bt* cotton. This could be a response of ladybeetles to the increased number of aphids on nontransgenic cottons.

Spiders tend to be very sensitive to most pesticides (4,18,20,21). Spider populations were significantly depressed by pesticide treatments on nontransgenic cotton and on *Bt* cotton in 1999 and 2001, probably due to a direct contact effect of insecticides. However, insecticides can also kill spiders by accumulating on their webs and poisoning them directly on the webs or repelling them from the webs, thereby exposing them to predation and contaminated leaf surfaces (16). There were more spiders on *Bt* cotton with no pesticide treatments than on nontransgenic cotton with no pesticides in 1999 and 2000. This could be due to the response of spiders to increased numbers of leafhoppers on *Bt* cotton and the establishment of their populations thereafter (data not presented here).

Any direct impact of transgenic *Bt* on predator populations is probably much weaker than that which would occur with insecticides; nevertheless, such tritrophic interactions are yet another factor that requires careful management in the increased deployment of *Bt*-transgenic crops. Potential impacts of *Bt* cotton on natural enemies include the removal of lepidopterous eggs, larvae and pupae as food sources for predators or as hosts for parasitoids. Clearly, within transgenic cotton fields, the abundance of some predators and parasitoids may be reduced, but this is unlikely to threaten their overall persistence in the cropping rotations that include cotton, since a significant proportion of *Helicoverpa* populations is always present on other crops (5,11). None of the known predators that attack Lepidoptera on cotton are specialists; *Helicoverpa* may be only an incidental prey for some key predators whose within-field abundance is maintained by other species of prey (7).

In conclusion, transgenic *Bt* cotton did not affect acarid mite populations and did not cause a considerable reduction in predator populations. Its effects on aphids were inconsistent between years. The use of *Bt* cotton did not lead to a reduction in total numbers of insecticide sprays in the course of 3 years because additional sprays were required against sucking pests. Pesticide applications reduced populations of spiders significantly on *Bt* cotton as well as on nontransgenic cotton. Therefore, the use of *Bt* cotton should be evaluated cautiously in China.

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