Transmission of *Bt* Toxin to the Predator *Propylaea japonica* (Coleoptera: Coccinellidae) Through Its Aphid Prey Feeding on Transgenic *Bt* Cotton

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ABSTRACT Laboratory feeding experiments using transgenic *Bacillus thuringiensis* (*Bt*) cotton plants were carried out to evaluate the transmission of *Bt* toxin among trophic levels and the effects of *Bt*-fed herbivorous prey on the coccinellid predator *Propylaea japonica* (Thunberg). The experimental host plants were transgenic *Bt*-expressing cotton cultivars, NuCOTN 33B and GK-12 and one corresponding untransformed isogenic (non-*Bt*) cultivar. The herbivorous prey, cotton aphid *Aphis gossypii* Glover, was not sensitive to *Bt* toxin. Trace amounts of *Bt* toxins (6.0 ng/g fresh mass [FM] in GK-12, 4.0 ng/g FM in NuCOTN 33B) were detected in *A. gossypii* feeding on *Bt* cotton cultivars. *Bt* toxin was detected in ladybirds preying on *Bt*-fed aphids, and its quantity increased as the predatory period extended (5–20 d). Small amounts of *Bt* toxin was also found in newly hatched, unfed coccinellid larvae when their parents fed on NuCOTN 33B-reared aphids (15.0 ng/g FM), but not when the parents were fed on GK-12–reared prey. In experiments assessing life history consequences, mortality was low (mean = 7.9%), confirming that the rearing methods were appropriate. There were no distinct differences in preimaginal mortality between predators reared on *Bt*-fed or *Bt*-free aphids. The preimaginal stages of the ladybird beetles developed faster when reared on prey fed on either *Bt*-cotton cultivar than those fed control prey. There was a trend of more adult malformations when the predator was fed with prey from one (GK-12) but not the other of the *Bt* cotton cultivars than on control prey. There were no significant differences in the preovipositing period or in fecundity. Ladybird beetles preying on *Bt*-reared aphids matured faster and mated more frequently than those fed on *Bt*-free aphids. These results indicate that *Bt* toxin expressed in transgenic cotton cultivars can be transmitted to a higher trophic level through a nontarget pest insect and may alter the biology and behavior of a predatory ladybird. Further work should evaluate the possible long-term, sublethal impacts on the agroenvironment under field conditions.

KEY WORDS transgenic *Bt* cotton, *Aphis gossypii*, *Propylaea japonica*, *Bt* toxin, transmission

ADOPTION RATES OF TRANSGENIC insect-resistant crops producing activated δ-endotoxins of the soil bacterium *Bacillus thuringiensis* (*Bt*) are among the highest for a new agricultural technology (James 2004). Transgenic plants produce the *B. thuringiensis* proteins in high doses and in most of their tissues throughout the season. This insecticidal toxin could become available to natural enemies in a new and modified form through nonsusceptible or sublethally affected nontarget herbivores prey feeding on these plants (Jepson et al. 1994). This could constitute an important pathway for the ecological impacts of transgenic plants (Andow and Hilbeck 2004), and several experiments have sought to test such potential impacts (for a review, see Lövei and Arpaia 2005).

One of the major genetically modified crops is cotton, and most of it is insect-resistant because of producing of *Bt* insecticidal protein (James 2004). China is one of the first countries in that commercialized *Bt* cotton developed to resist its major pest the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), was planted. *Bt* cotton was first commercially planted in northern China (Fang and Jia 1999). A substantial reduction of pesticide use in cotton growing has been achieved (Fang and Jia 1999, Zhang et al. 2000, Pray et al. 2002). However, the populations of several other pests, including the cotton aphid (*Aphis gossypii* Glover) (Homoptera: Aphididae), cotton whitefly [*Bemisia tabaci* (Gennadius)] (Homoptera: Aleyrodidae), and the green leaf bug (*Lygus lucorum* Mayer-Dűr) (Hemiptera: Miridae), have increased (Wilson et al. 1992, Fitt et al. 1994, Cui and Xia 1997, Zhang et al. 2000, Liu et al. 2002). This increases the importance of predatory natural ene-

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mies, further underlying the necessity to explore the potential nontarget impacts of *Bt* crops on them through the food chain.

The ladybird predator, *Propylaea japonica* (Thunberg) (Coleoptera: Coccinellidae), is a common, native insect species in China (Fang and Zhang 1998). Both the larvae and adults are natural enemies of aphids, thrips, and spider mites, as well as preying on eggs and young larvae of Lepidoptera (Fang and Zhang 1998). *P. japonica* does not avoid *Bt*-containing prey: in laboratory tests, newly hatched *H. armigera* larvae fed on transgenic *Bt*-cotton (Event 93-4) suffered higher predation rates by the coccinellids, *P. japonica* and *Coccinella septempunctata* L., than non–*Bt*-fed *H. armigera* larvae (Cui and Xia 1999). The impact of *Bt*-fed prey on predators is variable and unpredictable. The development of *Chrysopa sinica* Tjeder larvae is retarded, and the larval survivorship is decreased when fed with *B. tabaci* nymphs reared on transgenic *Bt* cotton NuCOTN 33B compared with rearing on non-*Bt* cotton cultivar Simian 3 (Guo et al. 2004). No differences were found on preimaginal development, larval survivorship, or adult body mass when *P. japonica* fed with *B. tabaci* nymphs reared on transgenic *Bt* cotton (Guo et al. 2004). However, adult survivorship of both *Bt-Bemisia* fed predators was lower, which may indicate that *B. tabaci* is not an optimal prey for either *C. sinica* or *P. japonica* (Guo et al. 2004). A different response may be obtained when the predator *P. japonica* is reared on *A. gossypii*, an optimal prey species.

The objectives of this study were (1) to investigate whether *A. gossypii* ingests *Bt* toxins when feeding on *Bt* cotton; (2) to establish whether *P. japonica* takes up *Bt* toxin when fed with cotton aphis reared on *Bt* cotton; and if it does, (3) to evaluate the toxic effects of *Bt* cotton on *P. japonica*, through its prey, *A. gossypii*.

Materials and Methods

Plant Material

The *Bt*-producing transgenic cotton cultivar, GK-12, expressing the combined Cry1Ac/Ab fusion gene (Xie et al. 1991) was provided by Liangshan Cotton Seed Company (Scientific Research Bases of Institute of Biotechnological Research of CAAS, Shandong Province, China). NuCOTN 33B expressing the Cry1Ac gene (Perlak et al. 1990) was provided by Monsanto and Hebei Jidai Cotton Seed Integrated Company (Shijiazhuang, Hebei Province, China). Simian 3, the corresponding isogenic nontransformed cultivar of GK-12, was provided by Siyang Cotton Raw Material Farm (Jiangsu Province, China).

Planting and Management

The seeds of Simian 3 and GK-12 were soaked in warm (50°C) water for ≈2 h before planting; seeds of NuCOTN 33B were sown directly. Two to three cotton seeds were sown in each pot (plastic pots, 110 by 90 mm in diameter, filled with nutrient solution-saturated vermiculite). Five trays (40 pots/tray) were sown for each cultivar. Seven to 10 d later, a new lot of cotton seeds were planted. All plants were fertilized with a nutrient solution [0.2 g KNO_3, 0.2 g KH_2PO_4, 0.8 g $FePO_4$, 0.1 g $Ca(NO_3)_2$, and 0.2 g $MgSO_4$, dissolved in 1,000 ml water] every 5–7 d. The *Bt* and non-*Bt* cultivars were kept in different greenhouses.

Insect Rearing Conditions

Aphis gossypii. Cotton aphids used in this study originated from adults and nymphs collected in a nontransgenic *Bt* cotton (Simian 3) field located at the Institute of Crop Germplasm Resources of CAAS, Beijing, between the end of July and early August. The aphids were kept on the cotton seedlings of the three cultivars, each for 30–50 d (four to seven generations). At 10- to 15-d intervals, new cotton plants were provided to ensure ad libitum prey for the experiments. Fifteen to 20 d later, when the population densities reached 30–50 individuals per leaf, the aphids were used as prey for *P. japonica.* The cotton aphids were reared on the three cotton cultivars in greenhouses (the *Bt* and non-*Bt* separately) set at identical conditions: 22.4 ± 1°C, 68.0 ± 5% RH, and a L:D photoperiod of 16:8 h.

Propylaea japonica. *Propylaea japonica* adults were collected in a maize field located in the Institute of Crop Breeding and Cultivation of CAAS in Beijing, China, at the end of September. Three adult pairs were caged in each insect rearing jar (cylindrical, 100 mm diameter, 160 mm tall). These beetles were fed with Chinese white poplar aphids, *Chaitophorus populialbae* (Boyer de Fonscolombe). In total, 60 male and female adults were reared in the growth room. Ten days later, eggs laid were collected in petri dishes (70 by 20 mm) and allowed to hatch at 26 ± 1°C.

Performance of *P. japonica*

The newly hatched coccinellid larvae (<12 h) were weighed individually on an electronic balance (0.01 mg precision) and then transferred with a fine brush onto a cotton leaf populated with 30–50 aphids. The young larvae were individually reared on the appropriate cotton plants in plastic containers (66 by 42 mm diameter, sealed with porous plastic wrap) at 25 ± 1°C, 70 ± 5% RH, and L:D photoperiod of 18:6 h. In total, 50, 51, and 51 *P. japonica* larvae were individually fed with aphids reared on the respective cotton cultivars Simian 3, GK-12, and NuCOTN 33B. *P. japonica* larvae were examined twice daily, and their development and mortality were recorded. Body masses of larvae in every instar, as well as adults, were measured on the first day of molting or on the day of eclosion, respectively. Freshly emerged adults were checked for malformations.

On the day of emergence, the newly emerged adults were paired randomly in a plastic container containing cotton leaf populated with plenty of aphid prey (>200 aphids). The status of mating and numbers of eggs laid by each female were recorded daily. The eggs laid on

the cotton leaf were removed every day, put in a petri dish, and allowed to hatch at 25°C. The petri dishes containing *P. japonica* eggs were changed at the same time. The eggs were checked daily, and the freshly emerged larvae were counted, weighed, and killed by freezing at −20°C. Twenty days later, the adults were also killed and kept at −20°C for further analysis. The frequencies of mating = [(numbers of mated/numbers of paired) × 100%] and oviposition = [(numbers of oviposition/numbers of mated) × 100%] were calculated. The premating and preovipositing periods were recorded.

In a different experiment, run concurrently, a further 40 newly hatched *P. japonica* larvae for each cotton cultivar were similarly fed with aphids until they developed into adults. Five, 10, and 15 d after emergence, 10 adults (5 females and 5 males) were preserved at −20°C, respectively, to test for *Bt* toxin content.

Propylaea japonica reared on aphids feeding on conventional Simian 3 cotton are hereafter referred to as "conventional ladybird beetles"; *P. japonica* reared on aphids feeding on *Bt* GK-12 cotton are hereafter referred to as "*Bt* GK ladybird beetles"; and *P. japonica* reared on aphids feeding on *Bt* NuCOTN 33B cotton are hereafter referred to as "*Bt* Nu ladybird beetles."

Bt Toxin Analysis

Cry1Ab or Cry1Ac protein levels in cotton plants, cotton aphids, and *P. japonica* were determined using a double sandwich ELISA kit (*Bt* Cry1Ab and Cry1Ac ELISA PathoScreen kits for respective *Bt* Cry1Ab and Cry1Ac endotoxins in plants; Agdia, U.S.A.) following the manufacturer's instructions. Cry1Ab and Cry1Ac standards (containing in respective Cry1Ab and Cry1Ac ELISA kits) at concentrations 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 ng/ml were used as calibrators, respectively. The amounts of the target proteins were measured by a microtiter plate reader (MR 550; Bio-Rad Laboratories, U.S.A.) at 630 nm together with above calibrations on each plate.

To quantify *Bt* toxin in the cotton aphid and the ladybird, protein was extracted from aphids reared either on transgenic *Bt* or non-*Bt* cotton cultivars or from ladybirds fed either on *Bt*- or non–*Bt*-reared *A. gossypii.* Ten samples from *A. gossypii* reared on the respective cotton cultivar and predator fed with the aphid reared on *Bt*- or non-*Bt* cotton cultivar were analyzed, respectively. The extraction procedures were as follows: 11.21 ± 0.23 (SE) mg (100 aphids) cotton aphid nymphs and adults were homogenized in 0.55 ml microsome extract buffer (0.4 g nonfat dried milk and 0.5 g Tween-20 in 100 ml phosphate buffered saline Tween-20 buffer) resulting in a 1:50 wt:vol dilution, centrifuged at 12 000 r.p.m. for 10 min, and 100 μl/well of undiluted supernatant was transferred onto enzyme linked immunoabsorbent assay (ELISA) plates. For *P. japonica*, 10 adults (5 males and 5 females) from each 5-, 10-, 15-, and 20-d-old fed with respective transgenic *Bt* or non-*Bt* cotton cultivar aphids from hatching were sampled, respectively. Single adults were weighed (5.06 ± 0.12 [SE] mg) and homogenized in 0.506 ml MEB buffer and centrifuged as above, and 100 μl/well of undiluted supernatant was transferred onto ELISA plates. Additionally, 10 samples of unfed neonates (10 neonates for each sample), hatched from the eggs laid by the above females, were tested for their *Bt* content. Neonates weighed 1.93 ± 0.11 (SD) mg, and they were homogenized in 0.386 ml MEB buffer amd centrifuged as above, and 100 μl/well of the undiluted supernatant was transferred onto ELISA plates.

To evaluate the producing of Cry1Ab or Cry1Ac in respective transgenic *Bt* cotton lines (GK-12 and NuCOTN 33B), 10 *Bt* plants each at five- to seven-developed-leaf stage were randomly selected, and 200-mg pieces from tender tips (two to three undeveloped leaves per tip, each at 20–30 mm diameter) were homogenized in 1 ml MEB buffer and centrifuged as above, and the supernatant was diluted 1:5 before transferring onto ELISA plates. Controls were 10 randomly selected plants of the conventional cotton line, Simian 3, prepared as above (except that these were not diluted).

Data Analysis

The differences in the amount of *Bt* toxin produced in the tender tips of the two transgenic *Bt* cotton cultivars and ingested by *A. gossypii* reared on respective *Bt* cotton cultivars were tested using Student's *t*-tests. One-way analysis of variance (ANOVA), with post hoc least significant difference (LSD) tests were conducted on the body mass of fourth-instar larvae and adults of *P. japonica*, on the *Bt* toxin content in different day-age adults and newly hatched larvae, on the age at first observed mating and preovipositing periods, and on the numbers of observed mating and numbers of eggs laid by individual female during egg laying period in the first 20 d. Nonparametric binomial tests were conducted on total developmental mortality and on frequencies of malformation, mated females, and ovipositing females. Nonparametric tests (*K* independent samples: Kruskal-Wallis *H*; two independent samples: Mann-Whitney *U* tests) were performed on the developmental duration of larva, pupa, and the total preimaginal period.

Calculations were performed using SPSS software (SPSS 1999).

Results

Transmission of *Bt* Toxin to Predator *P. japonica* Through *A. gossypii*

On average, 49.2 ng Cry1Ab/g plant fresh mass (FM) and 94.2 ng Cry1Ac/g plant FM were detected in the tender tips of transgenic GK-12 and NuCOTN 33B (both *Bt* cotton), respectively (Table 1). Adults and nymphs of *A. gossypii* contained 6.0 ng/g FM *Bt* toxin (no negative response in 10 tested samples) and 4.0 ng/g FM (four negative responses in 10 tested samples) when reared on GK-12 and NuCOTN 33B

Table 1. Mean ± SE Cry1Ab or Cry1Ac toxin content in tender tip (two to three undeveloped leaves per tip, each at 20- to 30-mm diameter) of *Bt* cotton and *A. gossypii* fed on transgenic *Bt* cotton

Cotton cultivar	*Bt* toxin content (ng/g FM)	
	Tender tip of cotton plants	*Aphis gossypii*
Simian 3 (non-*Bt*)	ND	ND
GK-12 (*Bt*)	49.2 ± 3.4*	6.0 ± 1.0
NuCOTN 33B (*Bt*)	94.2 ± 5.2	4.0 ± 1.5

The minimum detectable quantity of *Bt* toxin of the ELISA kits is 0.5 ng/g FM.

* Significantly different between two transgenic *Bt* cotton cultivars at $P < 0.01$ (Student's *t*-test).

ND, not detected.

cotton, respectively. No *Bt* toxin was detected in the tender tips of cultivar Simian 3 (control cotton) or in *A. gossypii* reared on this cultivar (Table 1).

The *Bt* toxin was passed on to the predator *P. japonica* through its prey reared on *Bt* cotton plants. When the adult fed for increasing lengths of time on *Bt* plant–associated prey, from 5 to 20 d, the amount of accumulated *Bt* toxin increased from 13.5 ng/g predator FM to 34.0 ng/g predator FM when fed GK-12–reared cotton aphids (5, 2, 1, and 0 negative responses in 10 respective tested samples of 5-, 10-, 15-, and 20-d-old the ladybird beetles), and from 9.0 to 24.0 ng/g when fed NuCOTN 33B–reared cotton aphids (6, 3, 1, and 0 negative responses in 10 respective tested samples of 5-, 10-, 15-, and 20-d-old the ladybird beetles; Fig. 1). In the case of NuCOTN 33B host plants, the *Bt* toxin was transmitted to progeny as well: newly hatched, unfed *P. japonica* larvae, originating from parents fed NuCOTN 33B–reared cotton aphids, contained 15.0 ng/g predator FM of *Bt* toxin (2 negative responses in 10 tested samples; Fig. 1). Trace amounts of the toxin were also detected in the non-*Bt* control samples of *P. japonica* adults (<6 ng/g predator FM; 1, 3, 6, and 2 positive responses in 10 tested samples of each tested day [5, 10, 15 and 20 d] adults), possibly because of contamination or a cross-reaction with other proteins (Dutton et al. 2002).

Effects of *Bt* Cotton on Development and Reproduction of *P. japonica*

Survival, mating behavior, and fecundity of *P. japonica* were not affected significantly when *Bt* cotton plants were used as a host plant for the prey aphids (Tables 2 and 3; Fig. 2). In contrast, the larval stage of conventional ladybird beetles was significantly shorter (6.6 d) than that of *Bt* GK ladybird beetles (7.1 d; Student's *t*-test: $t = 3.021$, df = 91, $P = 0.003$) but no different from that of *Bt* Nu ladybird beetles (6.5 d), even though conventional ladybird beetles matured slower than either *Bt* GK ladybird beetles or *Bt* Nu ladybird beetles (Table 2). Female *Bt* GK ladybird beetles were heavier than female *Bt* Nu ladybird beetles or conventional ladybird beetles (Fig. 2A). Second-instar male conventional ladybird beetles had a lower body mass than either *Bt* GK or *Bt* Nu ladybird beetles (Fig. 2B), but this difference was gone by the time the animals were adults, at which point there was a nonsignificant trend for male conventional ladybird beetles to have more mass than either *Bt* GK or *Bt* Nu ladybird beetles (Fig. 2B).

The proportions of mated female *Bt* Nu and *Bt* GK ladybird beetles that oviposited were lower than that of mated female conventional ladybird beetles (Simian 3: $n_{\text{oviposited}} = 10$, $n_{\text{mated}} = 12$; GK-12: $n_{\text{oviposited}} = 11$, $n_{\text{mated}} = 16$; NuCOTN 33B: $n_{\text{oviposited}} = 9$, $n_{\text{mated}} = 14$; binomial test, $P_{\text{GK-12 versus Simian 3}} = 0.121$, $P_{\text{NuCOTN 33B versus Simian 3}} = 0.074$; Table 3), even though female *Bt* Nu and *Bt* GK ladybird beetles engaged in significantly more matings and were 2 d younger than female conventional ladybird beetles when they first mated (Table 3). There was a trend for *Bt* GK ladybird beetles to suffer more malformations

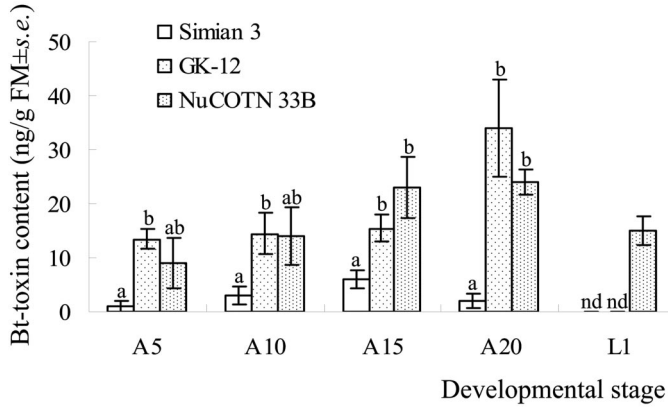

Fig. 1. Mean ± SE Cry1Ab or Cry1Ac toxin content in 5-, 10-, 15-, and 20-d-old *P. japonica* adults (A5, A10, A15, A20) fed with *A. gossypii* reared on *Bt* or non-*Bt* cotton plants throughout the entire adult life from hatching and in newly hatched *P. japonica* larvae (L1) produced by the above adults. Data within same group followed by different letters are significantly different at $P < 0.05$ (one-way ANOVA, LSD tests). n.d., not detected. The minimum detectable quantity of *Bt* toxin of the ELISA kits is 0.5 ng/g FM.

Table 2. Duration of development of different stages of *P. japonica* fed on cotton aphids reared on transgenic *Bt* and non-*Bt* cotton

Cotton cultivar	Development duration (d)[a]			Total developmental mortality (%)[b]
	Larval stage	Pupa	Hatching to eclosion	
Simian 3 (non-*Bt*)	6.6 ± 0.2a	4.6 ± 0.1a	11.2 ± 0.2a	10.0a
GK-12 (*Bt*)	7.1 ± 0.1b	3.5 ± 0.1b	10.6 ± 0.1b	7.8a
NuCOTN 33B (*Bt*)	6.5 ± 0.1a	3.8 ± 0.1c	10.3 ± 0.1b	5.9a

Data are means ± SE.
Means within a column followed by different letters are significantly different at $P < 0.05$.
[a] Nonparametric tests—*K* independent samples: Kruskal-Wallis *H;* two independent samples: Mann-Whitney *U* tests.
[b] Nonparametric tests: binomial test.

than conventional ladybird beetles (binomial test; $n_{normal} = 45$, $n_{malformed} = 2$; $P = 0.066$). No such trend was observed in *Bt* Nu ladybird beetles and conventional ladybird beetles ($n_{normal} = 47$, $n_{malformed} = 1$; $P = 0.488$; Table 3).

Discussion

A laboratory test is always an approximation of field conditions; thus, the first point is to consider how realistic our results are. We consider that the rearing conditions were appropriate, because our control mortality was at the very low end of reported values (Lövei and Arpaia 2005). Thus, despite elements of unrealistic conditions (unlimited prey, single prey type, no choice, uniform temperature) that are not common under field conditions (Lövei and Arpaia 2005), we believe the cases reported here can be expected to occur under field conditions.

Bt toxin was taken up from the transgenic cotton plants by *A. gossypii*, and this was detected in the aphid by ELISA (6.0 ng/g Cry1Ab [GK-12] and 4.0 ng/g Cry1Ac [NuCOTN 33B]; Table 1). Subsequently, the *Bt* toxins accumulated in the aphid gut and were thus delivered in increased concentration to the predatory ladybird *P. japonica.* An amount of 13.5–34.0 ng/g Cry1Ab (GK-12) or 9.0–24.0 ng/g Cry1Ac (NuCOTN 33B) toxin was detected in *P. japonica* adults (both *Bt* cotton cultivars) and even in their progeny (NuCOTN 33B only) when feeding on GK-12– and NuCOTN 33B–reared *A. gossypii* from hatching to eclosion (Fig. 1). The different performances between NuCOTN 33B and GK-12 on the tested ladybird beetle can be caused by more *Bt* toxin detected in NuCOTN 33B than in GK-12 cotton (Table 1) or by the plant background of both *Bt* cotton cultivars (Perlak et al. 1990, Xie et al. 1991).

The shortening of the premating period, trend of more malformed individuals, and (despite more matings) fewer ovipositing adults (Table 3) indicated that transgenic cotton, genetically engineered to produce the *Bt* toxin, may adversely affect the reproductive biology of this important natural enemy. The overall impact of these on the population dynamics of *P. japonica* and *A. gossypii* under agricultural conditions is difficult to assess. Malformation always results in unsuccessful matings (G.-F. Zhang, unpublished data), and unmated female individuals cannot lay any eggs (Lü et al. 1983). The adults of *P. japonica* can mate more than once during their lifetime, even though more eggs are produced when the females mate once versus several times (Wei and Ran 1983). Furthermore, the population of *P. japonica* could not increase if the adults mate but no eggs are produced.

Reduced body mass of *P. japonica* may be caused by suboptimal prey (indirect effects) or associated with *Bt*-related causes (direct effects) (Dutton et al. 2002). First-instar *Chrysoperla carnea* (Stephens) larvae have significantly reduced body mass when fed *Spodoptera littoralis* (Boisduval) larvae reared on *Bt* maize (Cry1Ab) (Dutton et al. 2002). Young *P. japonica* larvae also have lower body mass when fed for 72 h

Table 3. Effects of nontransgenic versus transgenic *Bt* cotton on developmental and reproductive characteristics of *P. japonica*

Cotton cultivar	No. of adults emerged (♀ + ♂)	Frequency of malformation (%)[a]	Frequency of mated females (%)[a]	Age at first observed mating (d)[b]	Preoviposition period (d)[b]	No. of observed matings/♀[b]	Frequency of ovipositing females (%)[a]	Fecundity (no. of eggs/♀)[b]
Simian 3 (non-*Bt*)	29+16	0a	75.0a	5.6 ± 0.3a	6.8 ± 0.4a	1.1 ± 0.1a	83.3a	27.7 ± 5.5a
GK-12 (*Bt*)	23+24	4.3a	69.6a	3.5 ± 0.3b	5.6 ± 0.5a	2.4 ± 0.4b	68.8a	16.8 ± 2.8a
NuCOTN 33B (*Bt*)	17+31	2.1a	82.4a	3.6 ± 0.6b	7.7 ± 1.7a	2.1 ± 0.3b	64.3a	33.0 ± 10.1a

Data are means ± SE.
Means within same columns followed by different letters are significantly different at $P < 0.05$.
Frequency of malformation = [(numbers of malformed)/(numbers of malformed + numbers of normal)] × 100%.
Frequency of mated females = [(numbers of mated)/(numbers of paired)] × 100%.
Frequency of ovipositing females = [(numbers of ovipositing female)/(numbers of mated female)] × 100%.
Fecundity = numbers of eggs produced by the adult during its egg laying peak period in the first 20 d.
[a] Nonparametric tests: binomial test.
[b] One-way ANOVA, LSD tests.

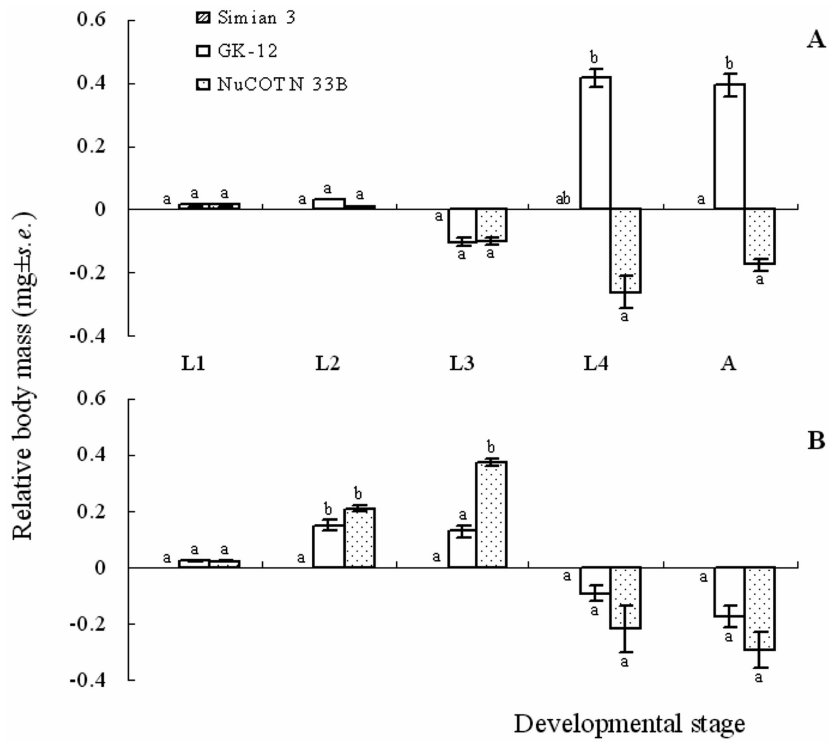

Fig. 2. Relative body mass (body mass of ladybird beetle reared with non–*Bt*-fed aphids minus that of reared with *Bt*-fed aphids) of four larval stages (L1, L2, L3, and L4) and adults (A) of *P. japonica* fed with *Bt* cotton (GK-12 and NuCOTN 33B)–fed aphids compared with non–*Bt* cotton (Simian 3)–fed aphids. Data within same developmental stage followed by different letters are significantly different at $P < 0.05$ (one-way ANOVA, LSD tests). A, female; B, male.

with 24-h-old NuCOTN 33B–reared *S. litura* (F.) larvae (Zhang et al. 2006). A combined interaction of poor prey quality and Cry1Ab or Cry1Ac may account for the negative effects. *C. carnea* larvae are not sensitive to Cry1Ab when sucrose solutions containing a range of Cry1Ab concentrations, reaching 0.1% mg/ml, are used as food of first-instar *C. carnea* (Romeis et al. 2004). Because *A. gossypii* is one of the optimal preys of *P. japonica* (Song et al. 1988, Fang and Zhang 1998), the reduced body mass found in this study, associated with the lines containing *Bt* toxin, may have been caused by food quality changes triggered by the host plant.

The presence of insect-resistant GM products (e.g., insecticidal proteins) does not necessarily cause detrimental effects, especially when nontarget herbivores are exposed to it. For example, *Bt* endotoxin-producing potatoes, genetically engineered for resistance to Colorado potato beetles [*Leptinotarsa decemlineata* (Say)], caused no detrimental effects on the survival, aphid consumption, development, or reproduction in the aphidophagous ladybird beetle, *Hippodamia convergens* (Guérin-Méneville), feeding on *Myzus persicae* (Sulzer) reared on the *Bt* potato plants (Dogan et al. 1996). *Bt* maize (producing Cry1Ab protein) caused no adverse effects on the survival, development, or mass of *C. carnea* after feeding on *Rhopalosiphum padi* L. (*Bt* toxin concentration 20 ng/g FM) and *Tetranychus urticae* (Koch) (*Bt* toxin concentration 2.5 μg/g FM) reared on the maize plants (Dutton et al. 2002). While it was suggested that the aphid does not take up the *Bt* toxin (Dutton et al. 2002), the presence of significant amounts of *Bt* toxin in spider mites (2.5 μg/g FM) indicates that the effect (or lack of effect) is not simply a function of the amount of toxin. *A. gossypii* feeding on *Bt* cotton contains similar amounts of *Bt* toxin (this study) to that of *R. padi* feeding on *Bt* maize (Dutton et al. 2002), yet the effects were different. The coccinellid predators in our experiments accumulated the toxin and may pass some of it on to their progeny. The consequences of such transmission are not yet known.

In some instances, however, negative effects can be found. *C. carnea* larvae, fed small larvae of *S. littoralis* or *Ostrinia nubilalis* (Hübner) reared on *Bt* maize (Cry1Ab), suffer a significant increase in mortality and a delay in development (Hilbeck et al. 1998). Similar effects, a significantly increased mortality, and a delay in development were found on *C. sinica* fed with *B. tabaci* reared on *Bt* cotton NuCOTN 33B (Guo et al. 2004). Usually, the lepidopterous larvae and *B. tabaci* are suboptimal prey for both lacewings and ladybird beetles (Fang and Zhang 1998), and the observed effects could indicate a combined effect of *Bt* exposure and nutritional deficiency (the "sick prey"

syndrome) (Hilbeck et al. 1998, Dutton et al. 2002, Guo et al. 2004, Zhang et al. 2006).

Furthermore, a recent study on *Bt* cotton DP99B and GK-12 (expressing the Cry1Ac gene and Cry1Ab/Ac fused gene, respectively), genetically engineered for resistance to *H. armigera*, has shown the potential for *Bt* toxicity to affect predatory lacewing larvae (*C. formosa* Brauer) through the nontarget aphid herbivore prey *A. gossypii* (Guo et al. 2005). Development was faster, but cocoon mass decreased when cultivar DP99B (*Bt*-expressing line)–reared *A. gossypii* were given to the predator; female longevity as well as egg viability also decreased (Guo et al. 2005). Peach-potato aphid (*M. persicae*) prey kept on transgenic potatoes expressing GNA (snowdrop lectin, *Galanthus nivalis* agglutinin, to make them resistant to the aphid) caused significantly reduced fecundity, egg viability, and longevity in the two-spot ladybirds *Adalia bipunctata* L. (Birch et al. 1999).

During the early season (i.e., from planting to the appearence of flower buds), the density as well as the population dynamics of predators in *Bt* cotton fields, Event Zhongmian 30 (producing Cry1Ac toxin) (Wan et al. 2002) and GK-12 (Wu and Guo 2003), are similar to those in conventional cotton fields. However, after pesticide spraying at the end of June, the numbers of predators decrease by 72.4–94.0% (Wan et al. 2002, Wu and Guo 2003) in conventional cotton fields compared with *Bt* fields. Spiders show similar effects (Liu et al. 2004). Overall, *Bt* cotton may have less negative influence on natural enemies than conventional cotton growing, at least in China.

However, our results on *P. japonica*, together with those on *C. formosa* (Guo et al. 2005), *C. carnea* (Hilbeck et al. 1998, Dutton et al. 2002) and *A. bipunctata* (Birch et al. 1999), suggest that tritrophic effects of antipest transgenes (also called plant incorporated protectants) on nontarget predators may be more widespread than previously assumed. Our laboratory findings have been confirmed by field data: *Bt* toxins (Cry1Ab or Cry1Ac) are present in *A. gossypii* nymphs and adults, *H. armigera* larvae, and even in *P. japonica* adults and larvae collected from *Bt* cotton fields (GK-12 and NuCOTN 33B) (Zhang et al. 2004). Our selected predator, *P. japonica*, feeds on aphids, spider mites, thrips, and also on lepidopteran eggs and young larvae in cotton fields (Fang and Zhang 1998). In a field situation, it might be unlikely that the predators complete their entire development by only feeding on one *Bt* cotton–related prey species. Nevertheless, further work should be performed on closely monitoring for possible longer-term, sublethal impacts of transgenic crops genetically engineered for insect resistance on the agroenvironment after commercial release.

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