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Crop Protection

Early instar response to plant-delivered Bt-toxin in a herbivore (*Spodoptera litura*) and a predator (*Propylaea japonica*)

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Abstract

Propylaea japonica is a significant predator in cotton fields in China. To assess the ecological effects of Bt-cotton cultivars (GK-12 and NuCOTN 33B, producing the fused Cry1Ab/Ac toxin and Cry1Ac toxin, respectively), the development of *Spodoptera litura* on transgenic Bt-cotton, the intake of Bt toxins, and the effects of Bt-cotton reared *S. litura* on young larvae of *P. japonica* were evaluated. Based on enzyme-linked immunosorbent assay (ELISA), the Bt-toxin concentrations in newly molted second-instar *S. litura* were 978.0 and 720.0 $ng\,g^{-1}$ when fed on GK-12 and NuCOTN 33B cotton, respectively. The survival rate of *S. litura* was decreased, the time required to reach the second-instar was prolonged, and the body mass was lowered, when reared on NuCOTN 33B compared to those on DPL5415. However, there were no significant differences between GK-12 and Simian 3 (Parental non-Bt-transgenic for GK-12). After feeding on GK-12 or NuCOTN 33B -fed *S. litura* neonates for 72 h, young larvae of *P. japonica* contained more Bt-toxin than cotton leaf tissue, but less than respective Bt-cotton fed *S. litura*. A decrease ($P = 0.061$) in body mass was observed when *P. japonica* young larvae were fed for 72 h with 24 h old NuCOTN 33B -reared *S. litura* larvae, compared to those fed with DPL5415 -reared *S. litura*; no significant differences were found when prey was fed with GK-12 vs. Simian 3 plants. Significantly fewer *P. japonica* larvae molted into second-instar when fed with *S. litura* reared on NuCOTN 33B, compared to ones fed prey from DPL5415 plants. No such effects were observed when feeding on GK-12 vs. Simian 3 -reared prey. These results suggested that the Cry1Ab/Ac fusion toxin had no direct effect on young larva of *P. japonica*, and a combined interaction of poor prey quality and Cry1Ac toxin may account for the negative effects observed on *P. japonica* development when fed NuCOTN 33B -reared *S. litura*.

1. Introduction

China is one of the largest producers of cotton (*Gossypium hirsutum* L.) in the world, and the cotton industry is an important part of the Chinese economy. Insect damage to cotton is significant. The most important pests are *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and *Aphis gossypii* Glover (Homoptera: Aphididae) (Guo, 1998). In China, *H. armigera* occurs mainly in the Yellow River (about 60% of the national acreage), Yangtze River (about 33%), Liao River, Xibei and South

China cotton-growing areas (Guo, 1998). In both the Yellow River and Yangtze River areas, *H. armigera* has 4–5 generations each year and is a significant pest of cotton, maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), soybean (*Glycine max* (L.) Merr.), tomato (*Lycopersicon esculentum* Mill.) and many other crops (Guo, 1997).

Since the mid-1990s, a new pest, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), had several outbreaks in cotton fields in Anhui, Jiangsu, Zhejiang and Hubei provinces (Xu, 1995; Qin et al., 2000; Liu, 2003). In the same areas, *S. litura* has 4–6 generations each year and is a serious pest of beans [soybean and wild soybean (*G. soja* Sieb. et Zucc.)], cotton and vegetables [cabbage *Brassica oleracea* var. *capitata* L. and Peking cabbage

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B. pekinensis (Lour.) Rupr.]. It can often cause enormous losses during warm and moist period, July to September (Zhu et al., 2001; Hu, 2003).

The ladybird *Propylaea japonica* (Thunberg) (Coleoptera: Coccinellidae) is a prominent natural enemy in cotton fields and mainly feeds on cotton aphis, spider mites, eggs and young larvae of lepidopteran pests (Fang and Zhang, 1998). The ladybeetle has 4–5 generations per year in northern China and 7–8 generations per year in southern China (Lü et al., 1983; Fang and Zhang, 1998). Because of its tolerance of higher temperature and preference for high humidity (>85% RH), *P. japonica* is the major predatory species in the Yangtze River cotton area from late July to September (Fang and Zhang, 1998).

Traditionally, cotton pests have been controlled by insecticides, first by chlorinated hydrocarbons (before the early 1980s), followed by organophosphates (from the mid-1980s), and pyrethroids (from the early 1990s). However, because of the development of resistance, from the mid 1990s, farmers resorted to mixtures of organophosphates, pyrethroids and whatever else they could obtain (Pray et al., 2002). Usually, 13–30 sprays were applied annually (Pray et al., 2001).

Transgenic Bt-cotton, engineered to continuously produce activated δ-endotoxins of the soil bacteria *Bacillus thuringiensis* Berliner (Bt), holds great promise in controlling *H. armigera* and other lepidopteran pests (Schuler et al., 1998). Adoption of transgenic Bt-cotton has been rapid in China since the first four Chinese Academy of Agricultural Sciences (CAAS) cultivars and one Monsanto cultivar were approved in 1997. From non-Bt-cotton in China in 1996, farmers planted 2.8 million ha of Bt-cotton in 2003 (Pray et al., 2001). In north China, over 90% of cotton was transgenic in 2001. The most widely planted Bt-cotton cultivars were different lines of the GK series (developed by CAAS) and NuCOTN 33B (developed by Monsanto, USA) (Pray et al., 2001; China Association for Science and Technology, 2003). Studies indicate that the main pest, *H. armigera*, as well as others, including *Anomis falva* Fabricius (Lepidoptera: Noctuidae) and *Ascotis selenaria* (Schiffermuller et Denis) (Lepidoptera: Geometridae), can be suppressed effectively (Cui and Xia, 1997a). However, Bt-cotton seems to be ineffective against *S. litura* and its population density and distribution area are increasing (Wang and Chen, 2002; Zhang et al., 2002; Deng et al., 2003). Meanwhile, in Bt-cotton fields, the population densities of *A. gossypii*, *Bemisia tabaci* (Gennadius) (Homoptera: Aleurodidae), *Thrips tabaci* L. (Thysanoptera: Thripidae), *Tetranychus urticae* Koch (Acarina: Tetranychidae) have increased, probably because of the substantial reduction in the use of pesticides (Cui and Xia, 1997a, 2000; Liu et al., 2002; Wang and Chen, 2002; Deng et al., 2003). This increases the potential role of natural enemies to keep these pests at tolerable densities. Consequently, it is important to study the impact of Bt-plants on these natural pest control agents. The field studies conducted to date with Bt-cotton have not shown any

significant negative effects on predator densities, including that of *P. japonica* (Cui and Xia, 1999; Zhang et al., 2000a). Occasionally, even a population increase in *P. japonica* was registered (Cui and Xia, 1997a, 2000; Liu et al., 2002; Deng et al., 2003). In laboratory bioassays, newly hatched *H. armigera* larvae were consumed by adults of several predators, including *P. japonica* (Cui and Xia 1997b, 1999). These results show that the studies conducted to date with Bt-cotton GK series have not shown any effect on *S. litura*; further research is essential to evaluate the relationships between Bt-toxin and the performance of *S. litura* and the fitness of its predator *P. japonica*.

Bt-toxins in spray formulations are considered compatible with natural enemies (Glare and O'Callaghan, 2000). Laboratory studies have indicated no detrimental effects of transgenic Bt-rice (expressing Cry1Ab or Cry1Ab/Ac fusion gene) or Bt-maize (expressing Cry1Ab gene) or Bt-cotton (expressing Cry1Ab/Ac fusion gene) on the survival and development of predators *Cyrtorhinus lividipennis* Reuter, *Orius majusculus* (Reuter) or *Chrysopa sinica* Tjeder when supplied with rice-fed or maize-fed or cotton-fed (Zwahlen et al., 2000; Bernal et al., 2002; Guo et al., 2004) piercing-sucking prey. Also, no detrimental effects were observed when *P. japonica* and *Coccinella septempunctata* L. adults feed on newly hatched *H. armigera* larvae reared on Bt-cotton (93-4, producing Cry1Ab/Ac fusion toxin) plants for 12 h (Cui and Xia, 1997b, 1999). However, leaf-chewing-prey feeding on Bt-transgenic maize increased the mortality of the predatory lacewing *Chrysoperla carnea* (Stephens) (Hilbeck et al., 1998; Dutton et al., 2002). However, a recent study strongly suggests that *C. carnea* larvae are not sensitive to Cry1Ab when sucrose solutions prepared containing a range of Cry1Ab concentrations, reaching 0.1% mg/ml, are used as food solution of first instar *C. carnea* (Romeis et al., 2004). Because first instars are much more sensitive to food quality than other instars or adults (Zalucki et al., 2001; Hódar et al., 2002; Fordyce and Shapiro, 2003), the survival rate of first instars is a crucial factor in determining the population size of an insect species (Hódar et al., 2002). Further work should emphasize on the performance of first-instar *P. japonica* fed with young leaf chewing prey reared on Bt-cotton plants.

The work reported here evaluated the development, mortality and body mass of secondary target lepidopteran species (*S. litura*), verified and quantified the presence of Bt-toxin in the herbivore and in the common predator *P. japonica* feeding upon the herbivore. Further, we evaluated the performance of *P. japonica* larvae consuming young *S. litura* larvae fed on either Bt-transgenic or non-Bt-cotton cultivars.

2. Material and methods

2.1. Cotton cultivars and growing conditions

We used two Bt-cotton cultivars, the Chinese-developed GK-12 and the Monsanto line NuCOTN-33B, and their

parent lines (Simian-3 and DPL5415, respectively, not produce Bt-toxin). Seeds of the cultivar GK-12, expressing the fused Cry1Ab/Ac gene (Xie et al., 1991), were provided by Liangshan Cotton Seed Company (Scientific Research Bases of the Institute of Bio-Technological Research of CAAS, Shandong Province, China). NuCOTN 33B, expressing the Cry1Ac gene (Perlak et al., 1990), was provided by Monsanto and Jidai Cotton Seed Integrated Company (Shijiazhuang, Hebei Province, China). Simian-3, the isogenic non-Bt-transgenic cultivar of GK-12, was provided by Siyang Cotton Raw Material Farm (Jiangsu Province, China) and DPL5415, the isogenic non-Bt-transgenic cultivar of NuCOTN 33B, by the Beijing Agency, Monsanto Fareast Ltd., USA.

The two transgenic Bt-cotton cultivars along with their corresponding parental lines were sown in a netted field (2.5 m in height, covered with wire cloth in 1×1 cm mesh) at the Institute of Crop Germplasm Resources of CAAS, Beijing, on 26 April. Twenty meters wide strips of vegetables (tomato, hot pepper, and tobacco) were planted between transgenic Bt- and non-Bt-cotton plots at the same time. Additionally, two rows of maize were planted between two parental lines and between the two transgenic Bt-cultivars. Each cotton cultivar consisted of 80 plants (four rows by 20 plants each). No pesticides were applied during the growing season. The plots were fertilized and irrigated as commonly practiced for commercialized cotton in the area.

2.2. Insects

Egg masses of *S. litura* were originally provided by Zhongshan University (Guangdong Province, China), where they were mass reared on artificial diet. In our laboratory, the larvae of *S. litura* have been reared on willowleaf least lettuce, *Lactuce saligna* L. forma *ruppiana* (Wallr.) Beck, for more than 10 generations. Rearing conditions were $27 \pm 1\,^{\circ}$C, $80 \pm 5\%$ RH, and L:D 16:8 h photoperiod.

Eggs of *H. armigera* were obtained from a laboratory colony reared on artificial diet (Liang et al., 1999) at the MOA Key Lab of Biological Control, for over 20 generations.

Larvae of *P. japonica* were collected from a maize field located at the CAAS Institute of Crop Breeding and Cultivation, Beijing, 4 km away from the cotton field, and were reared to adulthood on poplar aphids *Chaitophorus populialbae* (Boyer de Fonscolombe) (Homoptera: Aphididae). In total, 40 adults were obtained. Six newly emerged adults (3 females and 3 males) were kept in each container, and newly laid eggs were removed everyday.

2.3. Feeding test of S. litura on transgenic Bt- and non-Bt-cotton

Groups of 10 newly hatched *S. litura* larvae were transferred with a fine paintbrush into a plastic container

(36×88 mm diameter, sealed with porous plastic wrap). A newly developed cotton leaf, freshly taken from a plant of five to seven true leaf stage (wrapped with a piece of wet cotton to keep the leaf fresh), was placed in each container. The larvae were allowed to feed in a growth room at $27 \pm 1\,^{\circ}$C, $80 \pm 5\%$ RH, and L:D 16 : 8 h photoperiod. Thirty, 33, 30 and 34 replicates were used for GK-12, NuCOTN 33B, Simian-3 and DPL5415, respectively. Observations were made and the cotton leaf was replaced daily. Mortality and the duration of first stadium were recorded. One hundred newly molted, randomly selected second instars were weighed individually. Mean masses of the larvae were compared using one-way ANOVA with LSD procedures. Mean mortality and duration of the first stadium were calculated and tested using non-parametric tests to determine differences between treatments. For calculations, the SPSS program package was used (SPSS statistical package, SPSS Inc., 1999). In order to verify the biological activities of both Bt-cotton cultivars, similar bioassays of *H. armigera* also were conducted.

2.4. Bt-toxin analysis

Levels of Cry1Ab or Cry1Ac proteins in cotton leaves and in the herbivore and predator were determined using double sandwich ELISA kits (Bt-Cry1Ab and Cry1Ac ELISA Pathoscreen kits for Bt-Cry1Ab and Cry1Ac endotoxins in plants, respectively; Agdia Inc., USA) following the manufacturer's instructions. Cry1Ab or Cry1Ac standards (contained in the respective 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. The amounts of the target proteins were measured by a micro-titer plate reader (MR 550, Bio-Rad Laboratories, Inc., USA) at 630 nm.

To evaluate the produce of Cry1Ab or Cry1Ac in respective transgenic Bt-cotton lines (GK-12 and NuCOTN 33B), 10 Bt plants each at 5–7-leaf stage were randomly selected. From each plant, 200 mg pieces from newly developed leaves were homogenized in 1 ml MEB buffer (0.4 g non-fat dried milk and 0.5 g Tween-20 in 100 ml PBST buffer), centrifuged at 12000 rpm. for 10 min, and the supernatant diluted 1:5 before transferring onto ELISA plates. Controls were 10 randomly selected plants of the parent lines, Simian 3 and DPL5415, prepared as above (except that these were not diluted).

To quantify Bt-toxin in the two insect pests and the ladybird, protein was extracted from each pest reared either on transgenic Bt- or non-Bt-cotton cultivars, or from the ladybird fed either on Bt- or non-Bt-reared *S. litura*. Ten samples from each herbivore and carnivore species reared on each cotton cultivar or *S. litura* were analyzed. The extraction procedures were as follows: 3.5 ($\pm$0.2) mg of 24 h old *H. armigera* first instars were homogenized in 0.7 ml MEB buffer resulting in a 1:200 w/v dilution, centrifuged as above, and 100 μl/well of undiluted supernatant was transferred onto ELISA plates. Of *S. litura*, 6.6 ($\pm$0.3) mg newly molted second instars were homogenized

in 0.66 ml MEB buffer resulting in a 1:100 w/v dilution, centrifuged as above, and the supernatant was diluted with the same buffer at 1:2 before transferring onto ELISA plates. In the case of *P. japonica*, 1.24 (± 0.03) mg of 72 h old larvae were homogenized in 0.248 ml MEB buffer resulting in a 1:200 w/v dilution, centrifuged as above, and 100 μl/well undiluted supernatant was transferred onto ELISA plates.

For calibration and quantification, the dilutions of Cry1Ab or Cry1Ac were run in each ELISA test.

2.5. Performance of P. japonica

Freshly emerged *P. japonica* larvae (<4 h old) were individually fed twice daily with young *S. litura* larvae reared on either Bt- or non-Bt-cotton leaves for 24 h after hatching. Leaves of appropriate cotton cultivars were added daily to the plastic containers as a continuous food supply for *S. litura* larvae. The experiment was conducted three times with 20 ladybird larvae per treatment (totally, 60 individuals for each cotton cultivar treatment). Experiments were conducted at $26 \pm 1\,^\circ$C, $70 \pm 10\%$ RH, and L:D 16:8 h photoperiod. Development status and number of beetles molting to the second-instar were recorded daily. *P. japonica* larvae were weighed individually when 72 h old. Since there were no significant differences in body mass among the different repeats, the data of the three repeats were pooled and analyzed using one-way ANOVA with LSD procedures. The percentage of insects reaching the second-instar within 72 h was analyzed using non-parametric tests (SPSS statistical package, SPSS Inc., 1999).

3. Results

3.1. Performance of H. armigera and S. litura on transgenic Bt- and non-Bt-cotton

Compared with the parental cultivars, the body mass of *H. armigera* first instars reared on GK-12 and NuCOTN 33B for 24 h was 24.0% and 47.2% lower, respectively (Table 1). The mortality of the larvae reared on GK-12 over 48 h was almost five times that of the Simian 3 control and on NuCOTN 33B, mortality was even higher (Table 1). The mortality of the larvae fed on NuCOTN 33B was 78.0% higher than on GK-12.

There was no evidence of prolonged development time in *S. litura* first instars fed on GK-12 leaves compared to those on its parental cultivar Simian 3 (Table 2). The developmental period on NuCOTN 33B was 0.9 days longer than on its parental cultivar, DPL5415, and 0.7 and 0.6 days longer than those on Simian 3 and GK-12, respectively. These differences were significant (Table 2). The mortality of first instars reared on NuCOTN 33B was 70.9% higher than on cultivar DPL5415. There were no significant differences between mortality on cultivar GK-12 vs. cultivar Simian 3, or on GK-12 vs. DPL5415. The body mass upon reaching the second-instar reared on NuCOTN

Table 1
Mean mortality (% $\pm$ s.e.) of 48 h old and body mass (mg $\pm$ s.e.) of 24 h old first-instar *Helicoverpa armigera* reared on transgenic Bt- (GK-12 and NuCOTN 33B) and non-Bt (Simian 3 and DPL5415) cotton cultivars

Cotton cultivar	First-instar *H. armigera*	
	Body mass upon 24 h old (mg)[a]	Mortality upon 48 h old (%)[b]
Simian 3	0.25 ± 0.007 a	3.0 ± 1.1 a
GK-12	0.19 ± 0.006 b	15.3 ± 2.2 b
DPL5415	0.36 ± 0.012 c	5.6 ± 1.3 a
NuCOTN 33B	0.19 ± 0.007 b	69.7 ± 3.6 c

Means within a column followed by different letters are significantly different at $P < 0.01$.
[a] One-way ANOVA: LSD tests.
[b] Nonparametric tests, K independent samples: Kruskal-Wallis H and two independent samples: Mann-Whitney U tests.

Table 2
Mean mortality (% $\pm$ s.e.), developmental period (days $\pm$ s.e.) of first instars, and body mass (mg $\pm$ *s.e.*) of second-instar *Spodoptera litura*, reared on transgenic Bt- (GK-12 and NuCOTN 33B) and non-Bt (Simian 3 and DPL5415) cotton cultivars

Cotton cultivar	First instar[a]		Body mass upon reaching second instar (mg/larva)[b]
	Developmental period (days)	Mortality (%)	
Simian 3	3.6 ± 0.2 ac	5.3 ± 1.7 a	0.41 ± 0.01 a
GK-12	3.7 ± 0.1 a	9.0 ± 2.7 ab	0.40 ± 0.01 a
DPL5415	3.4 ± 0.1 c	5.1 ± 2.1 a	0.56 ± 0.02 b
NuCOTN 33B	4.3 ± 0.2 b	17.5 ± 3.4 b	0.38 ± 0.01 a

Means within a column followed by different letters are significantly different at $P < 0.05$.
[a] Nonparametric tests, K independent samples: Kruskal-Wallis H and two independent samples: Mann-Whitney U tests.
[b] One-way ANOVA: LSD tests.

33B was 0.18 mg lower than on DPL5415. Larvae grew slower on GK-12 than on DPL5415 (difference 0.16 mg) (Table 2).

3.2. Bt-toxin content of plants and insects

Mean concentrations of 50.0 ng Cry1Ab and 138.2 ng Cry1Ac toxins g^{-1} fresh Bt-cotton leaves were detected in cultivars GK-12 and NuCOTN 33B, respectively (Fig. 1). *H. armigera* first instars contained an average of 162.0 and 236.0 ng g^{-1} Bt-toxin when fed GK-12 and NuCOTN 33B, respectively, for 24 h. The Bt-toxin concentrations in *S. litura* second instars were 978.0 and 720.0 ng g^{-1} when fed on GK-12 and NuCOTN 33B cotton from their hatching, respectively. Small amounts of Cry1Ab or Cry1Ac were also detected in *H. armigera* (Cry1Ab, 18.0 ng g^{-1}; Cry1Ac, 40.0 ng g^{-1}) and *S. litura* (Cry1Ab, 64.0 ng g^{-1}; Cry1Ac,
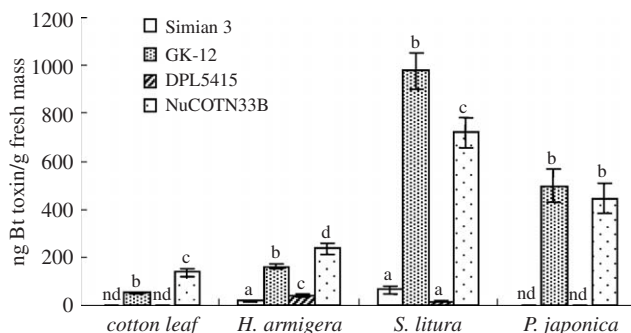
Fig. 1. Mean (±s.e.) Cry1Ab (Simian 3 and GK-12) or Cry1Ac (NuCOTN 33B and DPL5415) toxin concentrations in Bt- and non-Bt-cotton leaves, 24 h old *Helicoverpa armigera* (Hübner) larvae and second-instar *Spodoptera litura* (Fabricius) fed on Bt- and non-Bt-cotton, and in 72 h old *Propylaea japonica* larvae fed with 24 h old first-instar *S. litura* reared either on Bt- or non-Bt-cotton cultivars. Means within same group followed by different letters are significantly different at $P < 0.05$ (one-way ANOVA: LSD tests) (nd = not detected).

Table 3
Mean body mass (mg±s.e.) of 72 h old *Propylaea japonica* larvae and percentage reaching second instar (% ± s.e.) fed on first instar *Spodoptera litura* reared either on Bt-transgenic (GK-12, NuCOTN 33B) or on non-transgenic (Simian 3 and DPL5415) cotton cultivars

Cotton cultivar	Body mass (mg)[a]	% Molted into second-instar[b]
Simian 3	0.43 ± 0.03 ab	41.7 ± 4.4 a
GK-12	0.50 ± 0.04 a	66.7 ± 1.7 b
DPL5415	0.45 ± 0.03 ab	58.3 ± 7.3 b
NuCOTN 33B	0.37 ± 0.02 b	41.7 ± 1.7 a

Means within a column followed by different letters are significantly different at $P < 0.05$.
[a]One-way ANOVA: LSD tests.
[b]Nonparametric tests, K independent samples: Kruskal-Wallis H and two independent samples: Mann-Whitney U tests.

16.0 ng g^{-1}), when they fed on their respective parental cotton leaves, possibly due to the contamination or a cross-reaction with other protein (Dutton et al., 2002). After feeding on Bt plants for 24 h, *H. armigera* larvae contained more Bt-toxin than the leaf Bt-concentration ($P < 0.01$). When *S. litura* fed on GK-12 and NuCOTN 33B, respectively, the contents of Bt-toxin in the newly molted second instars were 18.6 and 4.2 times higher than the leaf Bt-concentration ($P < 0.001$).

Averages of 500.0 and 447.3 ng g^{-1} Cry1Ac toxins were detected in 72 h old *P. japonica* larvae when fed with 24 h old *S. litura* larvae reared on Bt-cotton, GK-12 and NuCOTN 33B, respectively, from their hatching. Also, the Bt-concentrations in 72 h old *P. japonica* larvae were higher than in the Bt-cotton leaf ($P < 0.01$) (Fig. 1).

3.3. Performance of P. japonica

Young larvae (72 h old) of *P. japonica* were 17.8% smaller than the control when fed with 24 h old *S. litura* first instars reared on Bt-cotton, NuCOTN 33B, although the differences were not significant ($P = 0.061$). Body mass of *P. japonica* fed on *S. litura* larvae reared on NuCOTN 33B was 26.0% less when compared with that on GK-12 ($P < 0.05$). When fed with *S. litura* larvae reared on NuCOTN 33B, fewer larvae successfully completed the first larval stage than the controls (Table 3). The body mass and percentage of development to second-instar were not different when the predator larvae were fed with *S. litura* larvae reared on GK-12 or on control cultivar (Table 3).

4. Discussion

The detection of Bt-toxin in *P. japonica* larvae fed with *S. litura* young larvae reared on Bt-cotton, GK-12 and NuCOTN 33B, indicated that predators feeding on young larvae of *S. litura* have been exposed to Bt-toxin from Bt-cotton. *P. japonica* larvae were lighter and suffered a delay in development when fed with *S. litura* larvae reared on NuCOTN 33B. Young *C. carnea* larvae fed with *S. littoralis* reared on Bt-maize (Cry1Ab) also showed lower body mass (Dutton et al., 2002). The further experiments demonstrated that Cry1Ab toxin has no direct effect on *C. carnea* larvae when high concentrations of the toxin directly were fed to the predator (Romeis et al., 2004). The prolonged development time and lower body mass of *S. litura* when feeding on Bt-cotton would suggest that the effect is not due merely to toxicity (if at all) but reduced prey quality (the "sick prey" phenomenon, Price et al., 1980). This could partly explain the negative effects observed on body mass of young *P. japonica* larvae when the predator was fed with this prey species reared on Bt-cotton cultivar, NuCOTN 33B. A similar reduction in prey quality may also occur in other noctuid cotton pests such as *H. armigera* and *H. punctigera* (Wallengren) (Fitt et al., 1994; Xing et al., 2001; Zhang et al., 2001), which develop more slowly, weigh less, and have a higher mortality rate when exposed to Bt-cotton.

The *S. litura* feeding experiments under controlled laboratory conditions showed that the activated Cry1Ac toxin expressed in NuCOTN 33B caused an increase in mortality, a delay in development, and a decrease in the body mass of first instar *S. litura* (Table 2). Larvae of the congeneric *S. littoralis* fed on transgenic Bt-maize plants containing the activated Cry1Ab toxin also show a higher mortality rate and delayed development (Dutton et al., 2002). The presence of Bt-toxin was confirmed by the ELISA tests of *S. litura* larvae. More Bt-toxin was ingested by *S. litura* reared on GK-12 than on NuCOTN 33B (Fig. 1), but without effects on the survival, development or body mass. The Cry1Ab/Ac fusion toxin was apparently not acutely toxic to this species possibly due to the fact that Cry1Ab/Ac fusion protein was not biologically active (Romeis et al., 2004). Guo et al. (2003) also stated that the

survival of *S. litura* larvae were not significantly affected when reared on GK-12 compared to parental line Simian 3.

Bt-cotton cultivars, GK-12 and NuCOTN 33B, caused higher mortality and lighter body mass in *H. armigera*, the target pest for Bt-cotton, as confirmed by previous studies (Xing et al., 2001; Zhang et al., 2000b, 2001). Moreover, the present study showed that the mortality of the young larvae reared on NuCOTN 33B was much higher than on GK-12 (Table 1), and the ELISA tests showed that more Bt-toxin present in *H. armigera* larvae reared on NuCOTN 33B than on GK-12 (Fig. 1). This could partially explain the detection of Bt-toxin in *P. japonica* collected in Bt-cotton fields in Nanpi, Hebei Province (Zhang et al., 2004).

The performing experiments of *P. japonica* larvae showed that Cry1Ac toxin (produced in NuCOTN 33B) mediated a poor prey-quality causes a decrease in body mass (although not significant, $P = 0.061$) and a delay in development (Table 3). Although the presence of Bt-toxin was confirmed by the ELISA tests in *P. japonica* larvae, there were no detrimental effects of GK-12 on body mass and development of *P. japonica*, or even shorter developmental period was observed (Table 3). An increased feeding was found in adults of *P. japonica* and *C. septempunctata* when fed with 12 h old *H. armigera* larvae reared on transgenic Bt-cotton plants (event 93–94) also containing the activated Cry1Ab/Ac fusion toxin (Cui and Xia, 1997b, 1999).

S. litura larvae accumulated more Cry1Ab or Cry1Ac than *H. armigera* (Fig. 1). Such differences between species are probably correlated with differences in their susceptibility to the toxins. Susceptible herbivores feed less and thus ingest fewer toxins (Head et al., 2001; Dutton et al., 2002). Both species still had more toxins in their bodies than the concentrations in plants. Consequently, more Bt-toxin was detected in *P. japonica* larvae fed with Bt-cotton reared *S. litura* than that in Bt-cotton leaves, since defecation takes place only after imaginal moulting, the toxin could not have been excreted (Romeis et al., 2004). The natural enemies, parasitoids and predators, of the pests, which are tolerant of the transgenic plants, are also potentially exposed to the transgene product when feeding on hosts in transgenic-crop fields (Schuler et al., 2001; Sétamou et al., 2002).

In the present study, the young larvae of *H. armigera*, *S. litura* and *P. japonica* gave different responses to Bt-cotton cultivars GK-12 and NuCOTN 33B when development, mass and mortality of these insects were evaluated (Tables 1–3). Higher efficiency was discovered on *H. armigera* but not on *S. litura*, since *H. armigera* is susceptible to Bt-cotton, the other pest caterpillars of genus *Spodoptera* such as, *S. litura*, are not fully susceptible and pests from other insect groups are not sensitive at all to the current Bt-cotton (Guo et al., 2003; Yang et al., 2005). And the different performances between NuCOTN 33B and GK-12 on the tested three insects, were tested and the effects were stronger on NuCOTN 33B than on GK-12. This can be due to more Bt-toxin detected in NuCOTN 33B than in GK-12 (Fig. 1), or due to the plant background of both Bt-cotton cultivars.

So far, observations on Bt-cotton fields have not shown significant detrimental effects on predators in Arizona (Wilson et al., 1992), Australia (Fitt et al., 1994) and Northern China (Cui and Xia, 1997a, 1999, 2000; Liu et al., 2002). *S. litura* is becoming a major problem in Bt-cotton fields in Southern China (Qin et al., 2000). Considering the importance of *P. japonica* in controlling of *S. litura* between July and September (Lü et al., 1983; Wei and Ran, 1983; Fang and Zhang, 1998), more detailed studies are needed to evaluate the effects of Bt-cotton on *P. japonica*.

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