

Effects of Bt-toxin Cry1Ac on *Propylaea japonica* Thunberg (Col., Coccinellidae) by feeding on Bt-treated Bt-resistant *Helicoverpa armigera* (Hübner) (Lep., Noctuidae) larvae

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Abstract: *Propylaea japonica* is an important predatory insect of common cotton pests. To assess the ecological effects of transgenic Bt cotton, expressing Cry1Ac toxin, on this predator, we examined the life history parameters of *P. japonica* for two generations by feeding them with Bt-resistant *Helicoverpa armigera*. After ingesting Bt-treated Bt-resistant *H. armigera* larvae in the third and fourth instar, the body mass and body length of adult *P. japonica* decreased, a combined effect of poor prey quality and Cry1Ac Bt-toxin may account for these effects. However, larval survivorship and development in these two instars, pupal mortality, fecundity and adult longevity of *P. japonica* were not affected in both the generations. These results suggest that ingesting Bt-toxin Cry1Ac-treated pests in advanced larval stage might have no significant effect on the fitness of predator *P. japonica*.

Key words: bionomics, Bt cotton, cotton bollworm, development, fitness, predator

1 Introduction

Bacillus thuringiensis (Berliner, Bt) is a naturally occurring rod-shaped soil-borne bacterium that is found worldwide. It produces a proteinaceous parasporal crystalline inclusion during sporulation. Upon ingestion by insects, this crystalline inclusion is solubilized in the midgut, releasing proteins called delta-endotoxins. These proteins (protoxins) are activated by midgut proteases and the activated toxins interact with the larval midgut epithelium to disrupt the membrane integrity, ultimately leading to insect death (Gill et al. 1992). Plant geneticists developed Bt cotton by inserting the genes for producing specific Bt protein into the cotton plant's own DNA. During the past several years, Bt cotton plantings in China have expanded quickly and Bt transgenic cotton is the main genetically modified organisms (GMO) crop variety in large-scale commercial production in China currently (Xue 2002; Huang 2004). Bt plants have a great potential in integrated pest management (IPM) programmes. They may be used to complement the effects of other biological control agents because of their higher selectivity than most insecticides (Blumberg et al. 1997). However, the impact of Bt transgenic plants on nontarget organisms is currently of concern, among which the effect of Bt on predators is becoming increasingly important in agro-ecosystems (Blumberg et al. 1997). Contrasting effects of Bt toxin on various

beneficial insects have been reported (Lövei and Arpaia 2005; O'Callaghan et al. 2005). Some studies have reported direct negative effects of Bt toxins on nontarget insects. The Bt Cry1Ab δ -endotoxin, which is expressed by most transgenic Bt maize plants, was reported to be toxic to *Chrysoperla carnea* (Stephens) (Hilbeck et al. 1998a). Further studies also showed that Bt toxin has acute toxic effects on *C. carnea* larvae when this toxin was incorporated into the artificial diet of its prey, *Spodoptera littoralis* (Hilbeck et al. 1999; Dutton et al. 2002). For coccinellids, Zhang et al. (in press) found that predator *Propylaea japonica* suffered increased larval mortality and decreased body mass after being fed on Bt-cotton-treated *Spodoptera litura*. However, results of no negative effects have also been reported in many studies. Pilcher et al. (1997) demonstrated that Bt corn pollen had no acute toxic effect on *Orius insidiosus* (Say), *C. carnea*, or *Coleomegilla maculata* (DeGeer). The same results were also found by Lundgren and Wiedenmann (2002) for *C. maculata*. Similarly, Al-Deeb et al. (2001) found no significant effect in the predator *O. insidiosus* when feeding on European corn borer larvae that had ingested a diet containing Bt toxins. Bt toxin was also found to have no adverse effect on ladybird beetles by Riddick and Barbosa (1998) and Sims (1995).

The conflicting results are probably because of the different methods used in the studies (Zwahlen et al.

2000; Lövei and Arpaia 2005) such as the different prey and varying forms and quantities of Bt toxin taken by the pests and predator. Aphids do not feed on plant cells but on phloem sap, in which Bt toxins are not expressed (Raps et al. 2001; Dutton et al. 2002). In Bt-susceptible pest larvae, Bt toxins are bound to receptors in the midgut epithelium, after which they are structurally rearranged (Masson et al. 1999), and most likely lose their toxicity to natural enemies like predators. In a lot of Bt-resistant larvae including *H. armigera*, Bt toxins lose the specific binding receptor in the midgut and cannot bind to midgut cells, and they may remain active and subsequently affect entomophagous natural enemies (Groot and Dicke 2002; Akhurst et al. 2003). Another important fact is that, Bt crops always affect natural enemies in two ways: (i) direct effects of Bt toxin and (ii) indirect effects of poor-quality prey or Bt toxin-ingested hosts. Unlike sensitive pests, Bt-resistant larvae can ingest enough Bt and allow large amount of Bt toxin to be passed on to higher trophic levels thus having direct effects on their predators or have less indirect effect on the predator because of undernourished pest larvae (Schuler et al. 2003). So the effects of Bt on predators can be measured more effectively and accurately by using Bt-resistant rather than Bt-susceptible pests. Furthermore, in laboratory and greenhouse tests, at least seven resistant laboratory strains of three pest species including *H. armigera* have completed their development on Bt crops (Tabashnik et al. 2003). If Bt-resistant cotton bollworm was found on Bt plants in the field, predators may be affected by the toxin. So using Bt-resistant pests to evaluate the direct effects of Bt toxins on natural enemies is an important and effective way to assess ecotoxicological risk (Schuler et al. 1999). In addition, most of the studies focused on the acute effects on non-target insects (Lövei and Arpaia 2005). Additional studies are needed to test for chronic effects over several generations before drawing a conclusion (Pilcher et al. 1997).

Helicoverpa armigera is an important insect pest of cotton and vegetable crops in Asia, Europe, Africa and Australia (Kaur et al. 2000). It is attacked by several endemic natural enemies, and one of the most important predator is *Propylaea japonica* Thunberg (Col., Coccinellidae) (Cui 1996; Fang and Zhang 1998). *P. japonica* is a polyphagous species and mainly feeds on cotton aphid, spider mites, and eggs and young larvae of lepidopteran pests (Fang and Zhang 1998). It is a prominent natural enemy of *H. armigera* in China, especially between mid-June and early July in northern China, while the second-generation *H. armigera* is present in the cotton field, although its primary prey is aphid (Song et al. 1988; Cui 1996). Furthermore, with the development of Bt-resistant pests in cotton field, more and more amount of Bt toxin will be passed on to higher trophic levels (Tabashnik et al. 2003). So the direct effects of Bt toxin on this predator is worth evaluating. We evaluated the acute and chronic effects of Bt toxin in *P. japonica* by examining their development, mortality, body mass and body length, adult longevity, and fecundity for two generations after being fed Bt-treated Bt-resistant cotton bollworm, *H. armigera* during the third and fourth instars.

2 Materials and Methods

2.1 Bt toxin and insects

The Bt toxin used in this study was provided by the Cotton Research Institute, Chinese Academy of Agricultural Sciences, as a mixture of spores and crystals of the HD-73 strain of *Bacillus thuringiensis* ssp. *kurstaki*, which contains 44% of Cry1Ac. The concentration of the Bt toxin in artificial diet used in the experiment is 30 µg/ml, which can kill 75% of susceptible *H. armigera* neonates. Exposure of *H. armigera* larvae to Bt toxin was accomplished by mixing the toxin with buffer (50 mmol/l Na₂CO₃) and incorporating it directly into the artificial diet (Wu and Gong 1997). The diet had cooled down to 50°C before the addition of Bt-buffer to prevent breakdown of the insecticidal material. After mixing, the experimental diet was thoroughly blended to ensure consistent concentration in the diet.

The Bt-resistant laboratory strain of *H. armigera* was provided by the Cotton Research Institute, Chinese Academy of Agricultural Sciences. This strain was maintained by feeding neonates with artificial diet in which Bt toxin had been mixed, and kept at 27 ± 1°C and 60% relative humidity (RH), under a 16 : 8 h (L : D) cycle. Mature caterpillars were allowed to pupate in moist soil substratum with a water content of 7% (soil was heated at temperature 120°C for 2 h and sifted through sieve with 36 apertures per cm²). Emerged adults were fed on 10% honey-water solution and allowed to mate for 2 days in fine-mesh cloth cages, and then transferred to laying containers for 3 days which were equipped with a window screen as the laying substratum. Eggs laid in the same day were collected and kept in glass jars for hatching.

Propylaea japonica adults were collected from the experimental maize fields of the Institute of Zoology, Beijing (39°92'N, 116°46'E), China in May 2003 and maintained in our laboratory for about eight generations at 26 ± 1°C with a 14 : 10 h (L : D) photoperiod on an unlimited supply of cotton aphid *Aphis gossypii* Glover. Fresh prey was offered daily to *P. japonica*.

2.2 Experimental design

Before this study, *P. japonica* was kept in the laboratory for four generations. Forty pairs of *P. japonica* were used in this study and every pair was kept in a separate container. Eggs laid by these 40 pairs were collected everyday and kept in the controlled environment chamber under the same conditions. After the eggs hatched, the first instars were moved to individual rearing containers. Fifty-sixty aphids of mixed stage were added to each rearing container every day. After 48 h of individual rearing, 210 early third instar *P. japonica* larvae were selected for this experiment. For *P. japonica* larvae, the quality of Bt-susceptible cotton bollworm larvae is similar to that of Bt-resistant larvae which contains no Bt (Shaoyan Zhang, Baoyu Xie, unpubl. data), and hence we divided these predator larvae into two treatments: (i) for feeding on Bt-resistant cotton bollworm larvae reared on Bt diet, (ii) for feeding on Bt-resistant cotton bollworm larvae reared on non-Bt diet.

The Bt-resistant *H. armigera* neonates hatching within 12 h were fed Bt diet or non-Bt diet for 24 h. Then, third instar *P. japonica* larvae were provided these larvae at a rate of 60 per day per *P. japonica* larva. At the same time, Bt or non-Bt cotton leaves were provided to every predator larvae. The containers were checked daily for removing excess *H. armigera* larvae and new cotton leaves provided. Predator larvae were left in the containers until the prepupal stage. Observations were made for every 6 h during this period.

When the predator larvae entered the prepupal stage, excess *H. armigera* larvae and cotton leaves were removed to prevent damage to pupae and fungal infections.

After the emergence of adults, sex ratio was recorded and adults were weighed with a digital balance ((Sartorius Ltd., Epsom, UK), 0.0001 g precision). Immediately after emergence, 15 males and 15 females of each treatment were anesthetized and measured under a dissecting microscope outfitted with a micrometer. Other adults in each treatment were fed with cotton aphids *ad libitum*. After 5 days, about 15 pairs of *P. japonica* in each treatment were selected randomly and allowed to mate in 65-cm dishes. We were certain that each pair of *P. japonica* adults mated because they were all under observation. After 24 h, adult males were removed. In each treatment, about 15 males and 15 females were also selected randomly and left unmated. Petri dishes which mated females stayed in were checked daily for eggs, females and excess food were removed to another new dish when oviposition was found. The old Petri dishes were checked daily for newly hatched larvae, and these larvae were removed into tubes individually after they had eaten their egg chorions. In this experiment, all adults were reared individually in 65-mm diameter Petri dishes. Every day, approximately 200 cotton aphids were offered as food to each predator adult in each treatment until its death.

For the second-generation experiment, we collected the first-generation eggs in two treatments separately about 20 days after mating. After these eggs molted, 105 newly hatched larvae from each treatment were selected as corresponding treatment for the second generation. The experiments in the second generation were conducted as in the first generation, including the diet patterns.

2.3 Statistical analysis

Data were analysed using SPSS package (SPSS 1998). Distributions of variables were tested for normality using the Kolmogorov–Smirnov test. Independent-sample *t*-test was used to test the difference between different treatments in same generation and different generations in the same treatment. Data of pupal mortality and sex ratio of *P. japonica* were tested by using chi-squared analysis, and only values of percentage are shown because there was no duplicate. Other values are expressed as mean \pm SE. Moreover, $P < 0.05$ was taken to be statistically significant.

3 Results

3.1 Effects of ingesting Bt-treated Bt-resistant cotton bollworm during the third and fourth instars on development, mortality and sex ratio of *P. japonica*

There is no effect on larval survivorship of predator *P. japonica* after they ingested Bt-treated Bt-resistant pest larvae during the third and fourth instars either in the first or second generation. All *P. japonica* larvae survived to the pupal stage in every treatment. No sexual difference between male and female was observed in the larval development time of the last two instars ($t = -0.675$, d.f. = 374, $P = 0.500$), pupal stage duration ($t = -0.726$, d.f. = 374, $P = 0.468$) and complete development period ($t = -1.062$, d.f. = 374, $P = 0.289$) of *P. japonica*, and hence we combined males and females in this experiment. Larval development in the last two instars of *P. japonica* was significantly slow in Bt-treated first-generation larvae ($t = -2.179$, d.f. = 165.078, $P = 0.031$). However, there is no effect on pupal stage duration and complete development period of *P. japonica* in both generations in Bt treatment (table 1). Pupal mortality of *P. japonica* in Bt treatment has no difference with that of control in two generations (table 1). Ingesting Bt-treated prey during the third and fourth instars also has no effect on the sex ratio of predator adults in both generations (table 1).

3.2 Effects of ingesting Bt-treated Bt-resistant cotton bollworm during the third and fourth instars on adult body mass and adult body length of *P. japonica*

There is a sexual difference in adult body mass and body length of *P. japonica* and female predators are heavier and longer than males (adult body mass, $t = -10.537$, d.f. = 360, $P = 0.000$; adult body length, $t = -9.948$, d.f. = 117, $P = 0.000$). Ingesting Bt-treated prey during the third and fourth instars can lessen the adult body mass and shorten the body length in male and female predators. In third and fourth

Table 1. Development (hours), larval mortality (%) and sex ratio (%) of *P. japonica* ingesting Bt-treated Bt-resistant cotton bollworm in the third and fourth larval stage

Treatment	<i>n</i>	Larval development time ¹	<i>n</i>	Pupal stage duration ²	<i>n</i>	Complete development period ³	<i>n</i>	Pupal mortality	<i>n</i>	% Female
First generation										
Control	103	97.95 \pm 0.89	91	82.38 \pm 0.73	91	227.84 \pm 1.23	104	12.50	91	51.65
Bt	104	101.83 \pm 1.54*	90	84.27 \pm 0.72 A	90	231.00 \pm 1.48 A	104	13.46	90	50.00
Second generation										
Control	112	98.14 \pm 1.22	94	80.52 \pm 0.82	94	224.62 \pm 1.21	112	16.07	94	53.19
Bt	110	100.04 \pm 1.28	102	81.29 \pm 0.84 B	102	227.29 \pm 1.17 B	110	8.18	101	45.55

Data are shown as mean \pm SE; *n* is number of replicate predators except pupal mortality and sex ratio.
Pupal mortality and sex ratio shown here in values of percentage because there was no duplicate.
*Following the mean values within a column indicate significant differences between treatments in the same generation ($P < 0.05$, *t*-test).
Different uppercase letters following the mean values within a column indicate significant differences between generations in the same treatment ($P < 0.05$, *t*-test).
¹Larval development time showed there was from early third instar to prepupae which ingested cotton bollworm larvae.
²Pupal stage duration was from prepupae to eclosion.
³Complete development period involved 48 h from molted to the third instar (which offered with *Aphis gossypii*), larval development time and pupal stage duration.

generations, body mass in Bt-treated predators was 4.88% and 11.72% lesser than that in controls in male adults and 1.64% and 5.91% lesser than that in controls in female adults, respectively, and significant effect of Bt treatment was found in the second generation in male and female adult body mass (male, $t = 5.146$, d.f. = 69.931, $P = 0.000$; female, $t = 2.273$, d.f. = 89, $P = 0.025$) (table 2). Correspondingly, adult body length was also shortened in male and female predators of Bt treatment and significant difference was found in adult males in the second generation ($t = 3.631$, d.f. = 28, $P = 0.001$) (table 2).

3.3 Effects of ingesting Bt-treated Bt-resistant cotton bollworm during the third and fourth instars on the fecundity and adult longevity of *P. japonica*

In *P. japonica*, Bt-treated prey had no effect on the number of eggs deposited and egg hatching percentage both in the first and second generations (table 3). Because adult longevity was different between sexes ($t = -3.609$, d.f. = 207, $P = 0.000$) and mated or unmated female adults ($t = 3.481$, d.f. = 104, $P = 0.001$), adult longevity of *P. japonica* was divided into four groups by sexes and mated or not. However, ingesting Bt-treated cotton bollworm larvae during the last two instars had no significant effect on mated or unmated adult males and females (table 3).

3.4 Generation differences of *P. japonica* fed Bt-treated Bt-resistant cotton bollworm during the third and fourth instars

Between generations, the development of *P. japonica* in Bt treatment was accelerated in the second generation and the changes were significant in pupal stage duration and complete development time (pupal stage duration, $t = 2.694$, d.f. = 188.38, $P = 0.008$; complete development time, $t = 1.988$, d.f. = 190, $P = 0.048$) (table 1). Adult body length and body mass of Bt-treated male *P. japonica* decreased significantly by 8.38% and 4.94%, respectively, separately in the second generation (adult body length, $t = 3.198$, d.f. = 28, $P = 0.003$; adult body mass, $t = 3.630$, d.f. = 77.54, $P = 0.001$), which was not observed in female *P. japonica* (table 2).

4 Discussion

Propylaea japonica was able to complete its development after ingesting Bt-treated Bt-resistant cotton bollworm larvae in the last two instars. Regardless of the decreased body mass and body length in adult males, ingesting Bt-treated prey in the third and fourth instars had a very limited effect on larval survivorship and development in these two instars, pupal mortality, fecundity, and adult longevity of *P. japonica*.

Table 2. Body mass and body length of *P. japonica* adults ingesting Bt-treated Bt-resistant cotton bollworm in the third and fourth larval stage

Treatment	Adult body mass (mg)				Adult body length (mm)			
	<i>n</i>	Male	<i>n</i>	Female	<i>n</i>	Male	<i>n</i>	Female
First generation								
Control	44	3.89 ± 0.07	47	4.28 ± 0.08	14	3.56 ± 0.05	15	3.84 ± 0.07
Bt	44	3.70 ± 0.07 A	45	4.21 ± 0.09	15	3.44 ± 0.04 A	15	3.71 ± 0.05
Second generation								
Control	40	3.84 ± 0.07	45	4.40 ± 0.08	15	3.46 ± 0.03	15	3.82 ± 0.05
Bt	51	3.39 ± 0.05* B	46	4.14 ± 0.08*	15	3.27 ± 0.04* B	15	3.74 ± 0.04

Data are shown as mean ± SE; *n* is number of replicate predators.
*Following the mean values within a column indicate significant differences between treatments in the same generation ($P < 0.05$, *t*-test). Different uppercase letters following the mean values within a column indicate significant differences between generations in the same treatment ($P < 0.05$, *t*-test).

Table 3. Fecundity and adult longevity of *P. japonica* ingesting Bt-treated Bt-resistant cotton bollworm in the third and fourth larval stage

Treatment	Male adult longevity (days)				Female adult longevity (days)				<i>n</i>	Number of eggs per female	<i>n</i>	Hatching percentage (%)
	<i>n</i>	Mated	<i>n</i>	Unmated	<i>n</i>	Mated	<i>n</i>	Unmated				
First generation												
Control	14	52.14 ± 4.71	9	49.22 ± 5.44	16	51.56 ± 5.19	12	64.50 ± 4.84	15	607.13 ± 88.79	15	33.59 ± 6.18
Bt	14	45.57 ± 5.47	10	47.40 ± 7.09	12	50.92 ± 4.54	10	64.40 ± 4.71	13	610.69 ± 73.29	13	42.05 ± 4.99
Second generation												
Control	16	45.25 ± 2.12	9	42.67 ± 3.23	12	48.67 ± 3.82	14	55.93 ± 4.39	10	507.60 ± 86.09	10	38.11 ± 5.77
Bt	13	48.23 ± 5.44	18	40.89 ± 2.93	14	44.64 ± 5.46	16	61.88 ± 5.95	11	521.55 ± 71.94	11	34.64 ± 6.44

Data are shown as mean ± SE; *n* is number of replicate predators.

4.1 Effects of ingesting Bt-treated Bt-resistant cotton bollworm during the third and fourth instars on development, mortality and sex ratio of *P. japonica*

After ingesting Bt-treated cotton bollworm larvae during the last two instars, no adverse effect in larval and pupal mortality of *P. japonica* was observed, which are similar to previous findings (Pilcher et al. 1997; Al-deeb et al. 2001). However, high larval mortality was found when this predator fed on Bt-treated Bt-susceptible *S. litura* (Zhang et al., in press). The quality of pests – which displayed slow development, high mortality and decreased body mass when fed on Bt-cotton – fed to the predator seems to be the main reason for these different results (Zhang et al., in press). But in the present study, the pests we used were Bt-resistant *H. armigera* larvae and no significant effect in their development and reproduction was found when fed on Bt-diet.

Larval development time of Bt-treated *P. japonica* in the last two instars was delayed in the first generation. Prolonged development time was also observed in *C. carnea* larvae reared on Bt-fed prey (Hilbeck et al. 1998b; Raps et al. 2001), and Hilbeck et al. (1998b) attributed this to the combined effect of Bt exposure and nutritional deficiency caused by sick prey. However, in this study, the prey we used was Bt-resistant cotton bollworm, and Bt diet had little effect on them. In addition, there was no difference in the last two instars' larval development time between two treatments in the second generation. Pupal stage duration and total development period of *P. japonica* were also not significantly different between treatments in both generations. Therefore, the only prolongation of *P. japonica* larval development time in Bt treatment in the first generation of this study might be induced by our insensitive observation. In our study, observations were made every 6 h to find new *P. japonica* prepupae. It seemed that this was not enough to find the exact prepupal time. Probably, because of this, the development time of larvae was prolonged and pupal stage duration shortened in Bt-treated first-generation *P. japonica*. However, the total development period of *P. japonica* should not be affected by this factor because it is the sum of larval and pupal stages.

4.2 Effects of ingesting Bt-treated Bt-resistant cotton bollworm during the third and fourth instars on adult body mass and adult body length of *P. japonica*

After ingesting Bt-treated cotton bollworm larvae during the two instar stages, *P. japonica* adults have a much lesser body mass and shorter body length than those of controls, especially in the second generation. Similar results in body mass were found in this predator when fed on Bt-cotton-treated *S. litura* larvae (Zhang et al., in press). Young *C. carnea* larvae fed *S. littoralis* reared on Bt-maize (Cry1Ab) also showed lower body mass (Dutton et al. 2002). Several factors might explain those negative effects observed in Bt-treated *P. japonica*. First, the quality of cotton bollworm larvae for *P. japonica* might reduce when they are reared on Bt diet. Although the prey we used

in this study was Bt-resistant cotton bollworm and their development/reproduction are completed well while reared on Bt diet, their nutrient content quality could have been affected a little by Bt dosing. Another possibility is that *P. japonica* may be susceptible to the Bt toxin, Cry1Ac. Direct negative effects of Bt-plants or Bt-toxin on predator have been reported in the predatory green lacewing *C. carnea* (Hilbeck et al. 1998a; Dutton et al. 2002). Whether the effects on the body mass and body length of *P. japonica* adults are induced by the quality of prey or directly by Bt toxin was not clear from this study. Further investigations should be conducted to verify this.

4.3 Effects of ingesting Bt-treated Bt-resistant cotton bollworm during the third and fourth instars on the fecundity and adult longevity of *P. japonica*

Although adverse effects had been found on adult body mass and body length of *P. japonica* after ingesting Bt-treated prey in the last two instars, neither the fecundity nor the longevity of this predator had been affected. Number of eggs per adult female, hatching percentage of eggs and adult longevity all had no difference between two treatments in both generations. The results agree with those of Al-Deeb et al. (2001) who found that feeding European corn borer larvae that had ingested a diet containing Bt toxins to the predator *O. insidiosus* did not cause significant adverse effect. Zhang et al. (2004) have also reported that there was no detrimental effect on development, survival and reproductive ability of the natural enemy *P. japonica* found in Bt cotton lines (NUCOTN 33B), although Bt toxin has been detected in their body. Furthermore, until now, no significant adverse effect was found on predators in Bt-cotton field studies in Arizona (Wilson et al. 1992), Australia (Fitt et al. 1994) and China (Li et al. 2002; Deng et al. 2003).

4.4 Generation differences of *P. japonica* fed Bt-treated Bt-resistant cotton bollworm during the third and fourth instars

Generation differences were found in pupal stage duration and complete development period of Bt treatment predators. The larval development was accelerated in the second generation, although there was no difference between Bt treatment and control in both generations. The shortened developmental stage can decrease the exposure of the predator to a disadvantageous situation, but it also might be the reason for the decreased body mass and body length of male predator adults in Bt treatment in the second generation. To certain degree, the reduction in adult body mass and body length may result in a reduced fitness in this predator (Song et al. 1988; Cui 1996). However, the adverse effects did not affect the fecundity and adult longevity in this study. The reduction of adult body mass and body weight in Bt treatment predators might be caused by the decreased quality of prey, or directly by Bt toxin or a combined effect of the two factors.

Our study showed that if large (third and fourth instar) larvae of *P. japonica* ingested Bt-treated Bt-resistant *H. armigera* larvae, larval survival in these two instars was not different from that of the control, and the resulting adults, while they had lower adult body mass and body size, did not have lower fecundity or egg viability. Before this study, another experiment which feeding first instar *P. japonica* larvae with *H. armigera* larvae was failed. High sensitivity of small predator larvae to Bt toxin and the different nutrient composition between cotton aphid and *H. armigera* larvae might be responsible for the results (Specty et al. 2003; Elizabeth et al. 2004). Similar to the small larvae, egg-laying adults were also kept on cotton aphid in this experiment. Other studies showed that the beetles were able to compensate for previous imbalances of proteins and lipid compositions (Mayntz et al. 2005). Further experiments need to be performed to find out the effects of feeding history of early instar and egg-laying adults on predator fitness.

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