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Biology of *Campoletis chlorideae* (Uchida) (Hym., Ichneumonidae) developing in Bt-treated, Bt-resistant *Helicoverpa armigera* (Hübner) (Lep., Noctuidae) larvae

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Abstract: Life history parameters in two generations of endoparasitoid *Campoletis chlorideae* (Uchida) were examined using *Bacillus thuringiensis* (Bt)-resistant *Helicoverpa armigera* (Hübner) larvae feeding on *B. thuringiensis* toxin Cry1Ac. In the laboratory, Bt toxin was fed to Bt-resistant host larvae continuously in case of Bt treatment and only before or after the host larvae were parasitized in Bt–P and P–Bt treatments, respectively. *C. chlorideae* pupae developed faster in Bt treatment than non-Bt treatment. The shortened pupal stage duration was mainly because of the feeding of host larvae on Bt-diet before being parasitized. Body length of adult male *C. chlorideae* developed inside Bt-treated Bt-resistant (Bt–Bt) *H. armigera* larvae significantly decreased, especially in host larvae feeding on Bt-diet after being parasitized. However, survival, pupal mortality and adult longevity of *C. chlorideae* were almost unaffected in Bt-resistant *H. armigera* larvae feeding on Bt-toxin. Furthermore, Bt-treated host larvae had the same effect on the F_1 progeny of *C. chlorideae* as the previous generation, and there was no significant difference between generations. This experiment suggests that there is very limited effect on the life history parameters in two generations of *C. chlorideae* parasitizing Bt–Bt *H. armigera* larvae. But both generations of *C. chlorideae* are affected when Bt-resistant *H. armigera* larvae fed on Bt toxin for different durations.

Key words: bionomics, Bt-cotton, cotton bollworm, development, host quality, parasitism

1 Introduction

The first insect-resistant transgenic crops expressing genes from *Bacillus thuringiensis* (Bt) are being grown commercially in a number of countries, and a range of transgenic plants expressing Bt are under development (Schuler et al. 2001). 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 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 parasitoids is becoming increasingly important in agro-ecosystems. Different effects of Bt on parasitoids of insect pests have been reported, and the results of the studies indicate negative effects to no effects and positive effects (Weseloh 1984a; Blumberg et al. 1997; Chilcutt and Tabashnik 1997; Atwood et al. 1998; Erb et al. 2001; Lövei and Arpaia 2005; O'Callaghan et al. 2005). The conflicting results are probably caused by the different methods used in the studies (Zwahlen et al. 2000), such as different hosts or varying period during which the hosts were reared on Bt diets (Lövei and Arpaia 2005).

In this study, we examined the tritrophic interactions among Bt toxin, Bt-resistant cotton bollworm *Helicoverpa armigera* (Hübner) (Lep., Noctuidae) and it larval endoparasitoid *Campoletis chlorideae* (Uchida) (Hym., Ichneumonidae). *H. 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 local species of natural enemies, one of the most important parasitoid being *C. chlorideae* (Blumberg et al. 1997; Kaur et al. 2000; Pandey et al. 2004).

In laboratory and greenhouse tests, at least seven resistant laboratory strains of three pest species including *H. armigera* have completed development on Bt crops (Tabashnik et al. 2003). The Bt crops do not preclude resistance problems in the future. Previous studies demonstrated that the percentage of parasitoids attracted to Bt-resistant larvae exposed on Bt-producing plants was much higher than Bt-susceptible larvae

exposed on Bt-producing plants (Schuler et al. 1999). Adult *Cotesia plutellae* females do not distinguish between Bt and wild-type oilseed rape plants and are more attracted to Bt plants damaged by Bt-resistant hosts than by susceptible hosts (Schuler et al. 2003). This stronger attraction to Bt plants damaged by resistant hosts was due to more extensive feeding damage (Schuler et al. 2003).

In Bt-susceptible larvae, Bt toxins are bound to receptors in the midgut epithelium, after which they are structurally rearranged (De Maagd et al. 1999; Masson et al. 1999) and thus most likely lose their toxicity to natural enemies. However, in Bt-resistant larvae, the toxins cannot bind to midgut receptor cells (Groot and Dicke 2002), as they may remain active and subsequently affect entomophagous natural enemies. Accordingly, if the Bt-resistant cotton bollworm was found on Bt plants in field, parasitoids will be attracted to them and may be affected by the toxin in these hosts. Furthermore, using Bt-resistant pests to evaluate direct effects of Bt toxins on parasitoid biology is an effective way to assess ecotoxicological risk (Schuler et al. 1999). Unlike sensitive hosts, Bt-resistant larvae can ingest enough Bt in their diet so that the effect on their parasitoids can be measured (Schuler et al. 2003). Bt-susceptible hosts always affect their parasitoids by their malnutrition. They even can perish along with their parasitoid (Blumberg et al. 1997). Using Bt-resistant pests is more effective and accurate to measure the effects of Bt on parasitoids than using susceptible hosts.

Periods of host larvae reared on Bt were inconsistent in related studies. In some studies host larvae contacted with Bt only before becoming parasitized (Weseloh and Andreadis 1982), others while parasitized (Atwood et al. 1997; Wallner et al. 1983) or after being parasitized (Atwood et al. 1997). Different results of these studies suggested that the periods host larvae contact with Bt might affect their parasitoids. Therefore, in this study, one experiment was conducted to determine the different effects on parasitoids while the host larvae ingested Bt diet before or after they were parasitized.

So a better understanding of the interaction between Bt, Bt-resistant pests and parasitoids is important to limit disruption of biological control, and to provide background knowledge essential for implementing measures for the conservation of parasitoid populations. All investigations in this study were carried out for two generations to test the sublethal or chronic and reproductive effects of Bt on the parasitoid, because sometimes the effects may be measurable only after exposure of the test animals to the toxin for several generations (Pilcher et al. 1997; Hilbeck et al. 2000).

2 Materials and Methods

2.1 Insects and Bt toxin

Campoletis chlorideae pupae were collected from *H. armigera* in Raoyang (38°14′N, 115°44′E), Hebei Province, China in 2001 and maintained in our laboratory for about 18 generations. A laboratory colony of susceptible *H. armigera* as host insects before experimentation was established by collecting full-grown larvae from the non-Bt cotton in Raoyang, Hebei Province, China in 2000. They were reared on artificial diet (Wu and Gong 1997) at 27 ± 1°C and a 16 : 8 h [light : dark (L : D)] photoperiod and they were not allowed contact with any insecticide, to maintain their susceptibility. Female wasps were 4–6 days old before they were provided with late second instar host larvae one by one, and the larvae were stung by mated female wasps one or two times. The parasitized host larvae were kept separately at 25 ± 1°C and a 14 : 10 h (L : D) photoperiod. After 6–7 days, the final instar parasitoid larvae emerged from the host and immediately pupated in a cocoon. The adult wasps were fed with a 10% honey–water solution and reared at 25 ± 1°C and a 14 : 10 h (L : D) photoperiod (Yin et al. 2003).

The Bt-resistant laboratory strain of *H. armigera* was provided by the Cotton Research Institute, Chinese Academy of Agricultural Sciences. Later in the study, this strain was maintained by feeding neonates with artificial diet into which Bt toxin had been mixed. Bt-resistant *H. armigera* was reared at 27 ± 1°C and 60% relative humidity (RH) with a 16 : 8 h (L : D) cycle. Adults were kept in fine mesh cloth cages and fed on 10% honey–water solution. Late second instar Bt-resistant *H. armigera* larvae were stung by mated female wasps in this study.

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 mM/l Na_2CO_3) and incorporating it directly into the artificial diet. The diet had to cool down to 50°C before addition of Bt-buffer to prevent breakdown of the insecticidal material (Cry1Ac protein). After mixing, the experimental diet was thoroughly blended to ensure consistent concentration in the diet.

2.2 Insect bioassays

The Bt-susceptible cotton bollworm neonates were provided as the controls and they were exposed to *C. chlorideae* females at late second instar. Bt-resistant neonates (< 12 h) were fed Bt or non-Bt diet, respectively. When they reached the late second instar stage, they were divided into following treatments.

(A) Exposure of undosed larvae to *C. chlorideae* (P, $n = 40$).
(B) Exposure of Bt-dosed larvae to *C. chlorideae* (Bt, $n = 40$).
(C) Bt dosing after exposure to *C. chlorideae* and non-Bt diet given before parasitization (P–Bt, $n = 40$).
(D) Bt dosing before exposure to *C. chlorideae* and non-Bt diet given after parasitization (Bt–P, $n = 40$).

The experiment was replicated three times for control and other four treatments. The fates and performances of *C. chlorideae* in Bt–Bt cotton bollworm were examined by treatments control, A and B, and the relationship between this fate and performance and the timing of Bt dosing (before or after parasitization) was examined by treatments control, A, C and D. For each replication of the first generation, 200 (40 × 5) late second instar cotton bollworm larvae were parasitized by 30 female *C. chlorideae* parasitoids one by one.

The 90 (30 × 3) parasitoids used in this experiment were all aged 4–6 days, satiated (when an insect no longer accepts the offered food, after a period of active feeding) with 10% honey solution, mated and experienced (the females that had already parasitized 10–20 hosts) females (Pandey et al. 2004). Larvae assigned to the Bt–P treatment were fed on the Bt diet for 4 days – from hatching until they reached late second instar. Larvae in the P–Bt treatment were fed on the Bt diet for about 6 days – from parasitization to pupation of parasitoids. Larvae assigned to Bt treatment were allowed to feed on the Bt diet continuously for about 10 days from hatching of hosts to pupation of parasitoids. We were certain that all larvae were parasitized in the experiment, because they were all were under observation, and 10 larvae per treatment were randomly selected and dissected, which showed them all to be parasitized. Following parasitism, larvae were maintained on diet until emergence and the pupation of parasitoid larvae or death of the *H. armigera* larvae. Daily observations were made during this period. Bt or non-Bt diet was replaced every 3 days. Diet was removed from the tubes on the day of pupation to prevent fungal infections and pupae were weighed (Sartorius, 0.0001 g precision (Sartorius Ltd., Epsom, UK)), 2 days after pupation. Emerging adults were fed with a 10% honey–water solution. In each treatment, 15 males were anaesthetized and measured under a dissecting microscope outfitted with a micrometer on the day of their eclosion. Pupal mortality and longevity of males and females were recorded.

The adults of same treatment were allowed to mate (female : male; 1 : 1.5) for 24 h on the second day after eclosion. Mated female wasps were provided with differently treated host larvae 4–6 days after eclosion, and host larvae were also divided into five groups: control, P, Bt, Bt–P and P–Bt. The experiments on the second generation were conducted as in the first generation.

2.3 Statistical analysis

Data were analysed using the SPSS package (SPSS 1998). Distributions of variables were tested for normality using the Kolmogorov–Smirnov test. For *C. chlorideae*, differences in larval development time, pupal stage duration and adult longevity were analysed by *K*-independent samples (Kruskal–Wallis) in non-parametric tests among treatments in the same generation. The differences of these parameters were analysed by two-independent samples (Mann–Whitney) in non-parametric tests between generations. Other parameters among treatments in same generation were analysed by ANOVA followed by LSD test; independent-sample *t*-test was used to test the difference in these between generations. Data in percentages were transformed by either the arcsine and square-root transformation. All values are expressed as mean ± SE. $P < 0.05$ was taken to be statistically significant.

3 Results

3.1 Larval development time and mortality of *C. chlorideae* developing in Bt–Bt *H. armigera*

There was no difference in larval development time (from day of larviposition to pupation) and mortality between control (only parasitized in susceptible hosts) and P treatment (only parasitized in Bt-resistant hosts) (table 1). Larval development time of Bt treatment was significantly shortened in the first generation compared with that of control and P treatment ($P = 0.003$ and 0.015, respectively). In Bt–P treatment, larval development time was shorter than that in control, P and P–Bt treatments in both generations (G1: $P = 0.010$, 0.049 and 0.005; G2, $P = 0.000$, 0.000 and 0.002, respectively) (table 1).

Ingestion of Bt toxin by *H. armigera* larvae and the duration of the larvae ingesting Bt did not affect the mortality of *C. chlorideae* larvae in the first generation (table 1). There was also no significant difference in parasitoid larval mortality between Bt and control in the second generation, although mortality in Bt treatment was higher than that in P treatment (table 1).

Table 1. *Larval development time, larval mortality and pupal stage duration, pupal mortality, pupal mass of* Campoletis chlorideae *developing in Bt-resistant* Helicoverpa armigera *larvae fed on Bt toxin (Cry1Ac)*

Treatment	*n*	Larval development time (days)	*n*	Larval mortality (%)	*n*	Pupal stage duration (days)	*n*	Pupal mortality (%)	*n*	Pupal mass (mg)
First generation										
Control	81	7.98 ± 0.07 a	120	32.50 ± 2.50	71	6.70 ± 0.08 a	81	12.19 ± 2.94 ab	32	10.26 ± 0.47 a
P	81	7.93 ± 0.08 a	119	31.97 ± 6.10	74	6.65 ± 0.10 a	81	9.32 ± 3.39 ab	32	9.29 ± 0.36 ab
Bt	72	7.81 ± 0.14 b	120	43.33 ± 2.20	62	5.95 ± 0.06 bA	68	9.11 ± 4.57 abA	29	8.57 ± 0.25 b
P–Bt	67	8.03 ± 0.09 aA	117	43.45 ± 7.07	67	6.57 ± 0.08 a	66	1.28 ± 1.2 b	25	8.29 ± 0.32 b
Bt–P	76	7.75 ± 0.10 b	118	33.12 ± 4.63	66	6.11 ± 0.10 bA	79	17.67 ± 8.84 a	34	9.42 ± 0.40 ab
Second generation										
Control	84	8.00 ± 0.08 a	118	28.78 ± 2.00 bc	74	6.80 ± 0.08 a	84	13.10 ± 2.39 bc	25	10.05 ± 0.43 a
P	88	7.94 ± 0.08 a	120	26.67 ± 3.00 c	75	6.80 ± 0.07 a	88	14.62 ± 7.70 bc	30	9.27 ± 0.27 ac
Bt	73	7.92 ± 0.13 a	119	36.97 ± 1.54 ab	55	6.55 ± 0.10 bB	75	26.75 ± 3.85 abB	23	8.74 ± 0.36 bc
P–Bt	91	7.78 ± 0.06 aB	120	24.17 ± 8.33 c	85	6.77 ± 0.07 a	91	7.63 ± 2.80 c	28	8.04 ± 0.30 b
Bt–P	73	7.48 ± 0.10 b	119	38.70 ± 5.61 a	52	6.46 ± 0.11 bB	73	29.26 ± 3.67 a	14	9.42 ± 0.60 ac

Different lowercase letters following the mean values within a column indicate significant differences among treatments in the same generation ($P < 0.05$).
Different uppercase letters following the mean values within a column indicate significant differences between generations in the same treatment ($P < 0.05$).
Significant differences found in larval mortality, pupal mortality and pupal mass were analysed by ANOVA followed by LSD test among treatments in the same generation and by independent-sample *t*-test between generations.
Significant differences found in larval development time, pupal stage duration were measured by K-independent-samples (Kruskal–Wallis) in non-parametric tests among treatments in the same generation and by two-independent-samples (Mann–Whitney) in non-parametric tests between generations.

However, mortality of parasitoid larvae in Bt–P treatment was significantly higher than that of control, P and P–Bt treatments in the second generation (P = 0.046, 0.020 and 0.008, respectively) (table 1). Mortality was measured 13 days after treatment, which was a sufficient time for the larvae to pupate.

3.2 Pupal stage duration, pupal mass and mortality of *C. chlorideae* developing in Bt–Bt *H. armigera*

The data of pupal stage duration and pupal mass for male and female parasitiods were combined because there was no difference between sexes. Pupal stage duration of *C. chlorideae* in Bt treatment was shortened than that in control and P treatment in both generations (G1: P = 0.000 and 0.000; G2, P = 0.022 and 0.010, respectively) (table 1). Pupal stage duration in Bt–P treatment differed significantly from control, P and P–Bt treatments in both generations (G1: P = 0.000, 0.000 and 0.000; G2: P = 0.015, 0.006 and 0.011, respectively) (table 1).

The parasitoid pupae in the Bt group weighed less than that in the control and P groups and significant difference was observed when compared with control group (G1: P = 0.007; G2: P = 0.013) (table 1). Pupal mass of parasitoids in P–Bt treatment was significantly decreased compared with control in both generations (G1: P = 0.002; G2: P = 0.000) and with P and Bt–P treatments in the second generation (P = 0.010 and 0.020, respectively). Pupae from P–Bt larvae were 1.97, 1 and 1.13 mg lighter than control, P and Bt–P treatments in the first generation, and 2.01, 1.23 and 1.38 mg lighter in the second generation, respectively (table 1).

There was no difference in pupal mortality among control, P and Bt treatments in both generations. Pupal mortality of parasitoid in Bt–P treatment was higher than that in P–Bt treatment in both generations (G1: P = 0.040; G2: P = 0.007). Pupal mortality in Bt–P treatment was also higher than that in control and P treatments in the second generation (P = 0.029 and 0.044, respectively; table 1).

3.3 Adult male body length and adult longevity of *C. chlorideae* developing in Bt–Bt *H. armigera*

The body length of male parasitoids in Bt treatment were found to be reduced significantly compared with that of control and P treatments in both generations (G1: P = 0.000 and 0.000; G2, P = 0.000 and 0.008; table 2). Adult body length of male parasitoids was also shorter in Bt–P and P–Bt treatments than that in control and P teatments for both generations (table 2).

Female parasitoids lived 2–6 days longer than males (P = 0.000). Adult longevity for females and males of Bt treatment has no difference with that of control and P treatments in both generations (table 2). The longevity of *C. chlorideae* female and adult males in Bt–P and P–Bt treatments also had no difference with control and P treatments in both generations except the female adults in P–Bt treatment in the first generation (table 2).

3.4 Generation differences of *C. chlorideae* developing in Bt–Bt *H. armigera*

In the second generation, larval development time in P–Bt treatment was shortened by 3.11% than that in the first generation, but there were no differences among P–Bt, control and P treatments within both generations (table 1). Pupal stage durations for Bt and Bt–P treatment in the second generation were significant longer than those in the first generation (P = 0.000 and 0.035, respectively).

No difference was found in pupal mass of parasitoids in all treatments between generations. This was also the case with adult body length except P–Bt treatment. Our

Table 2. *Body length and longevity of* Campoletis chlorideae *adult developing in Bt-resistant* Helicoverpa armigera *larvae fed on Bt toxin (Cry1Ac)*

			Adult longevity (days)			
Treatment	*n*	Adult body length (mm)	*n*	Female	*n*	Male
First generation						
Control	15	5.90 ± 0.08 a	12	13.83 ± 0.99 a	24	11.33 ± 0.55 ab
P	15	5.64 ± 0.07 a	10	15.30 ± 1.02 a	21	11.10 ± 0.84 ab
Bt	15	5.11 ± 0.15 b	23	15.83 ± 1.19 a	20	13.95 ± 1.15 a
P–Bt	15	5.34 ± 0.08 bA	8	11.13 ± 0.55 bA	19	9.47 ± 0.60 bA
Bt–P	15	5.16 ± 0.10 b	16	14.44 ± 1.32 a	30	13.57 ± 0.93 aA
Second generation						
Control	15	5.67 ± 0.09 a	14	15.14 ± 1.37	20	12.85 ± 0.70 ab
P	15	5.43 ± 0.08 ac	26	17.92 ± 0.91	33	13.03 ± 0.78 ab
Bt	14	5.07 ± 0.09 b	16	18.00 ± 1.64	24	14.00 ± 1.16 ab
P–Bt	15	5.01 ± 0.11 bB	29	18.41 ± 1.21 B	29	11.83 ± 0.69 bB
Bt–P	15	5.23 ± 0.92 bc	12	17.00 ± 1.85	14	17.14 ± 1.86 aB

Different lowercase letters following the means within a column indicate significant differences among treatments in the same generation ($P < 0.05$).
Different uppercase letters following the means within a column indicate significant differences between generations in the same treatment ($P < 0.05$).
Significant differences found in adult body length were analysed by ANOVA followed by LSD test among treatments in the same generation and by independent-sample *t*-test between generations.
Significant differences found in adult longevity were measured by *K*-independent samples (Kruskal–Wallis) in non-parametric tests among treatments in the same generation and by two-independent samples (Mann–Whitney) in non-parametric tests between generations.

results also indicate increased pupal mortality in Bt treatment in the second generation ($P = 0.042$; table 1), but there was no difference in pupal mortality among Bt, control and P groups within generations.

Longevity of adult females in P–Bt treatment was prolonged in the second generation ($P = 0.004$). The same trend was found in the longevity of adult males of P–Bt and Bt–P treatments in the second generation (table 2), but no difference was found in the longevity of adult males of these two groups compared with control and P treatments within generations (table 2).

4 Discussion

Campoletis chlorideae was able to complete its larval development in Bt-resistant cotton bollworm larvae. Regardless of the accelerated larval and pupal development or shortened body length, *H. armigera* larvae feeding on Bt diet have a very limited effect on their parasitoid, *C. chlorideae*. The Bt dosing period could significantly affect the larval and pupal development, pupal mortality and adult longevity of parasitoids.

4.1 Larval development time, pupal stage duration, and larval and pupal mortality of *C. chlorideae* developing in Bt–Bt *H. armigera*

Ahmad et al. (1978) reported that larval development of *Apanteles melanoscelus* was slowed in Bt-treated hosts. Extended larval development time was also found in *Compsilura concinnata*, a tachinid parasitoid, but only in superparasitized *Lymantria dispar* (Lep., Lymantriidae) (Erb et al. 2001). Erb et al. (2001) suggest that individual parasitoid development may have been limited by factors such as food availability. The parasitoid larvae may have to prolong their development time because of the Bt-induced nutritional deficiencies of host larvae.

However, Bt toxin may have little effect on the hosts when they develop resistance to Bt. Our data showed that host larvae fed on Bt-containing diets can shorten the development time of parasitoid larvae, especially when they fed on Bt before being parasitized. A similar effect was found for pupal stage duration – it was found to be accelerated in Bt treatment and this change was also mainly because of the feeding of the host larvae with Bt before parasitization. The results reported here support Weseloh's (1984b) conclusion that the larval development of *C. concinnata* was accelerated in Bt-treated hosts. Sun et al. (2003) also demonstrated that pupal stage duration of parasitoid *C. chlorideae* in Bt-treated cotton bollworm larvae was shortened. Weseloh (1984b) suggested that *C. concinnata* may be triggered to emerge from hosts when levels of juvenile hormones in the host are low, and those of ecdysone are high, which occurs just before the ecdysis of larvae. In addition, transferring to non-Bt food suddenly after being parasitized may be another reason for accelerated larval and pupal development in Bt–P treatment, which may give appetite to the host larvae and result in nice growth of the hosts and their parasitoids to induce the shortened larvae development time and pupal stage duration of *C. chlorideae*. In any case, the shortened larval development time and pupal stage in our study indicate that immature parasitoids require less time to develop completely, thus decreasing the exposure of host and parasitoid to predation and superparasitization. This situation may also reduce the risk of disadvantageous weather.

4.2 Pupal mass and male adult body length for *C. chlorideae* developing in Bt–Bt *H. armigera*

The pupal mass and body length of adult males in Bt treatment were found to be decreased which are similar to the findings of Erb et al. (2001) with *L. dispar* and its parasitoid *C. concinnata*. Decreased pupal mass was also found in *C. chlorideae* development in Bt-treated cotton bollworm (Sun et al. 2003; Ren et al. 2004). In this experiment, the development of larvae and pupae has been found to be accelerated in Bt–P treatment, but there was little difference in pupal mass among this treatment, control and P. In contrast, pupal mass and adult body length were found to be decreased in P–Bt treatment even though the development duration of larvae and pupae are not different from that of control. Thus, the shortened larval development time and pupal stage duration are unlikely to be responsible for the reduction in pupal mass and length of *C. chlorideae* adult males which develop in Bt-treated host larvae, in this study. Our results also indicated that feeding Bt toxin to host larvae after parasitization (P–Bt) may be the primary reason for the decreased pupal mass and adult body length. Baur and Boethel (2003) reported that increased stress by the developing parasitoid larvae may have caused host larvae to become more susceptible to Bt toxin. Erb et al. (2001) also found that parasitization by *C. concinnata* followed by Bt treatment (P–Bt) kills more gypsy moth larvae than either Bt treatment followed by parasitism (Bt–P) or parasitized alone. Cotton bollworm larvae fed the Bt toxin after parasitization probably become less adaptive to the Bt diet though they are Bt-resistant. This may induce the parasitoid developing in these host larvae to have decreased pupal mass and adult male body length. It is also possible that Bt toxin affected the parasitoids directly because pupal mass and adult body length of the three Bt treatments (Bt, Bt–P and P–Bt) were all found to be decreased compared with control in this experiment, especially in adult body length. The reduction in pupal mass or adult body length of parasitoids may result in reduced fitness of adults (Erb et al. 2001). However, in this study, the adverse effects of Bt on pupal mass and adult body length did not affect either the pupal mortality and longevity of adults (table 2) or the life history parameters of their offspring generation.

4.3 Adult longevity of *C. chlorideae* developing in Bt–Bt *H. armigera*

Our results show that only female longevity of P–Bt treatment in the first generation was shorter than control and P treatments. Our finding was consistent with previous studies in *C. chlorideae* with sublethal

dose of Bt on cotton bollworm (Sun et al. 2003) and in other parasitoids (Salama et al. 1991; Baur and Boethel 2003). Our results implied that the shortened longevity of parasitoids developing in P–Bt-treated host larvae in the first generation may be induced by their host larvae which develop under double pressure: parasitization and Bt toxin. However, adult longevity in P–Bt treatment was prolonged for both sexes in the second generation. In addition, the longevity of parasitoids in Bt treatment was not shortened in both generations. It can therefore be concluded that developing in Bt-treated hosts has little effect on the longevity of *C. chlorideae* adults.

4.4 Generation differences of *C. chlorideae* developing in Bt–Bt *H. armigera*

In the second generation, parasitoids in P–Bt treatment have an accelerated larval development and a shortened male adult body length than in the first generation. Although the adult size of parasitoids can influence fitness such as adult longevity (Prütz and Dettner 2004), the adult longevity of parasitoids in P–Bt treatment has not been shortened but prolonged in the second generation, both in adult males and females in our study. Longer pupal duration and higher pupal mortality were found in Bt treatment in the second generation. We were not sure if there was any relationship between the changes of these two parameters but we found that the changes between generations did not affect the difference among Bt, control and P treatments within the first or second generation. Although the mortality was higher in the second generation, pupal mortality of Bt treatment had no difference from control in both generations. Pupal stage duration of Bt treatment was shorter than control in each generation, although it was prolonged in the second generation. The same conditions were found in pupal stage duration and adult male longevity of Bt–P treatment. Furthermore, the studies on the effect of Bt on parasitoids for more than one generation are rare presently. Therefore, we can only conclude that there was no adverse chronic effect when *C. chlorideae* developed in Bt–Bt cotton bollworm.

In summary, our study showed that *C. chlorideae* was significantly affected by the different durations of Bt-resistant *H. armigera* larvae feeding on Bt toxin. However, the results of parasitoid development in Bt–Bt larvae in this study demonstrated that *C. chlorideae* can completely develop in Bt-treated cotton bollworm larvae and Bt toxin has only a limited effect on the life history parameters in the current generation and in next generation. A primary conclusion that can be drawn is that Bt-resistant cotton bollworm in Bt cotton fields is a suitable host for parasitoid *C. chlorideae*. Further studies are needed in this regard.

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