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Effects of *Bacillus thuringiensis* δ-endotoxin-fed *Helicoverpa armigera* on the survival and development of the parasitoid *Campoletis chlorideae*

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Abstract

With the deployment of transgenic crops expressing δ-endotoxins from *Bacillus thuringiensis* (Bt) for pest management, there is a need to generate information on the interaction of crop pests with their natural enemies that are important for regulation of pest populations. Therefore, we studied the effects of the Bt δ-endotoxins Cry1Ab and Cry1Ac on the survival and development of the parasitoid *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) reared on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae fed on Bt toxin-intoxicated artificial diet. The *H. armigera* larvae fed on artificial diet impregnated with Cry1Ab and Cry1Ac at LC_{50} (effective concentration to kill 50% of the neonate *H. armigera* larvae) and ED_{50} (effective concentration to cause a 50% reduction in larval weight) levels before and after parasitization resulted in a significant reduction in cocoon formation and adult emergence of *C. chlorideae*. Larval period of the parasitoid was prolonged by 2 days when fed on Bt-intoxicated larvae. No adverse effects were observed on female fecundity. The observed effects appeared to be indirect in nature, because no Bt proteins were detected through enzyme-linked immunosorbent assay in the *C. chlorideae* larvae, cocoons, or adults fed on Cry1Ab- or Cry1Ac-treated *H. armigera* larvae. The effects of Bt toxin proteins on *C. chlorideae* were due to early mortality of *H. armigera* larvae, that is, before completion of parasitoid larval development.

Introduction

The cotton bollworm/legume pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is the most important constraint to increasing production and productivity of crops worldwide. It is widely distributed in Asia, Africa, Australia, and southern Europe (IIE, 1992), and causes annual loss of over $2 billion in the semiarid tropics, despite application of insecticides costing over $500 million annually (Sharma, 2005). *Bacillus thuringiensis* (Bt) (Berliner) has been used extensively for the management of lepidopteran insect pests, particularly *H. armigera*, in India, China, Philippines, Malaysia, and North America (Gujar, 2005). Significant progress has also been made over the past two decades in handling and introduction of novel genes into crop plants to impart resistance to biotic and abiotic stresses, and to improve nutrition. Genes from Bt have been deployed successfully for pest control through transgenic crops on a commercial scale (Hilder & Boulter, 1999; Sharma et al., 2001; Shelton et al., 2002). Cry genes from *B. thuringiensis* conferring resistance to insect pests have been inserted into several crops, such as maize, cotton, potato, tobacco, rice, broccoli, chickpea, pigeonpea, cowpea, and soybean. In 2005, Bt-transgenic cotton and maize varieties were grown on more than 23 million ha worldwide (James, 2005). To ensure a sustainable deployment of transgenic insect-resistant plants, it is important that they should be compatible with other control methods, including biological control agents that are of importance for natural regulation of pest populations. *Bacillus thuringiensis* proteins are considered to be environmentally benign and their use will avoid the hazards associated with the use of synthetic insecticides for pest management.

The solitary endoparasitoid *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) is an important biocontrol agent of *H. armigera* larvae in pigeonpea, chickpea, and cotton (Patel & Patel, 1972; Bhatnagar et al., 1982; Kumar et al., 1994; Romeis & Shanower, 1996). It

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preferentially attacks second- and third-instar hosts. Parasitoid larvae emerge from the fourth-instar hosts to pupate and spin a cocoon (Nikam & Gaikwad, 1989). The total developmental period of *C. chlorideae* lasts for about 15–16 days (Nandihalli & Lee, 1995). We studied the effects of Bt-intoxicated *H. armigera* larvae on the host-specific parasitoid, *C. chlorideae*, under laboratory conditions. Such information is necessary to develop appropriate strategies for development and deployment of transgenic crops for pest management and sustainable crop production.

Materials and methods

Insect culture

The *H. armigera* larvae were reared on chickpea-based semisynthetic artificial diet under laboratory conditions (27 ± 2 °C and 65–85% r.h.) (Armes et al., 1992). The larvae were first reared in groups of 150–200 in 250 ml plastic cups containing a 2–3 mm thick layer of artificial diet. After 5 days, when the larvae became cannibalistic, they were transferred to 10-well plates with a single larva per well. Each well (3 cm in diameter × 2.5 cm in depth) contained 5–7 ml of artificial diet, on which the larvae were allowed to feed until pupation. After emergence, 10 pairs of moths were released in wooden cages (30 × 30 × 30 cm) and fed on 10% sucrose solution. Nappy liners with a rough surface were placed inside the cage for oviposition. The *H. armigera* culture maintained in the laboratory was used for rearing *C. chlorideae* and for conducting the bioassays.

The cocoons of *C. chlorideae* were collected from chickpea fields at the ICRISAT research station, Patancheru, India, and placed individually in plastic vials (15-ml capacity) for adult emergence. The adult wasps were released in plastic cages (2-l capacity) for mating, and were fed with 10% honey solution. For oviposition, the mated females were transferred to 50-ml Plexiglas vials placed in an inverted position on a Petri dish. Single second instars of *H. armigera* were offered to the parasitoid females for oviposition. The parasitoid females, in general, parasitized the *H. armigera* larvae within 1–2 min. After parasitization, the *H. armigera* larvae were removed and placed on artificial diet for further development. The culture was maintained at 27 ± 2 °C and 65–75% r.h. in the laboratory.

Intrinsic toxicity of Bt proteins to *Helicoverpa armigera*

Bacillus thuringiensis ssp. *kurstaki* δ-endotoxins Cry1Ab and Cry1Ac (obtained from Dr. Marianne P. Carey, Case Western Reserve University, Department of Biochemistry, Cleveland, OH, USA) were used to determine LC_{50} (effective concentration to kill 50% of the neonate *H. armigera* larvae) and ED_{50} (effective concentration to cause a 50% reduction in larval weight) dosages. Stock solutions of Cry1Ac and Cry1Ab were prepared by dissolving the proteins in distilled water. Subsequently, various volumes of the solution were mixed into *H. armigera* diet with a magnetic stirrer to obtain six serial dilutions both for Cry1Ac (having 13.30, 6.65, 3.33, 1.66, 0.83, and 0.42 µg of Cry1Ac per ml diet) and Cry1Ab (having 26.60, 13.30, 6.65, 3.33, 1.66, and 0.83 µg of Cry1Ab per ml diet). One and a half ml of this diet was dispensed in each well of a 10-well plate. One neonate *H. armigera* larva was released in each well. Each treatment (dilution) had 10 larvae per replication, and there were three replications in a completely randomized design. Observations were recorded on larval mortality and weight of the surviving larvae after 5 days of feeding on the artificial diet treated with the various dilutions of the Bt proteins. One set of larvae was fed on untreated artificial diet as a control. The mortality and weights of *H. armigera* larvae fed on control diet were considered as the basis on which the LC_{50} and ED_{50} values were calculated for the larvae fed with Bt-treated diets. The LC_{50} and ED_{50} values were calculated using the logdose-probit analysis (Chi, 1997).

Host-mediated effects on *Campoletis chlorideae*

The effects of Cry1Ab and Cry1Ac proteins on the biology of *C. chlorideae* were studied by feeding *H. armigera* larvae on artificial diet at LC_{50} and ED_{50} levels (as per the values obtained above). One cm^2 piece of diet was provided to each *H. armigera* larva, which was kept individually in 15 ml plastic vials to avoid cannibalism. The vials were secured with cotton plugs. The *H. armigera* larvae were reared on artificial diet with or without Cry1Ab or Cry1Ac proteins at the LC_{50} and ED_{50} levels for 24 h, and then exposed to *C. chlorideae* females for parasitization. Before parasitization, the *C. chlorideae* females were allowed to mate for 3 days inside a cage (30 × 30 × 30 cm), and provided with 10% (wt/vol) honey solution in a cotton swab as a food source. A total of 12 *C. chlorideae* females were used to parasitize the host larvae reared on four different treatments (– –, + –, – +, and + +) (see below), three females were used in each treatment. Each parasitoid female parasitized an equal number (15) of *H. armigera* larvae. After parasitization, the *H. armigera* larvae were transferred to the artificial diet with or without Bt proteins. The experiments were conducted in a completely randomized design, with a total of 45 larvae per treatment in three replications to evaluate the effects of Bt protein-treated *H. armigera* larvae on *C. chlorideae*. The following four treatment combinations for each Bt protein (Cry1Ab and Cry1Ac) and concentration (LC_{50} and ED_{50}) were included: (+ –) = the *H. armigera* larvae first fed with the

Bt artificial diet for 24 h before parasitization, and then fed with Bt-free control diet until cocoon formation; (– +) = the *H. armigera* larvae fed with control diet before parasitization, and then with Bt diet for 24 h; (+ +) = the *H. armigera* larvae fed with Bt diet for 24 h before and after parasitization; and (– –) = the *H. armigera* larvae fed with Bt-free control diet before and after parasitization.

Observations

Parasitized *H. armigera* larvae were checked every day and observations were recorded on the following parameters: larval mortality, parasitoid cocoon formation, days to cocoon formation (egg + larval development period), pupal period, adult emergence, adult weight, sex ratio, and fecundity of the *C. chlorideae* females from different treatments. For the fecundity test, three randomly selected *C. chlorideae* adult pairs obtained from each treatment (including control) were released inside a cage ($30 \times 30 \times 30$ cm), and the females were allowed to mate for 3 days. The adults were provided with 10% honey solution in a cotton swab as a food source. After 3 days of mating, each female was provided with *H. armigera* larvae up to their daily parasitization capacity. Parasitization of *H. armigera* larvae with these females was continued until they died. Total number of *H. armigera* larvae parasitized by a female in its lifetime was recorded as fecundity/female.

Detection of Bt proteins in *Helicoverpa armigera* and *Campoletis chlorideae*

After feeding the *H. armigera* larvae on Bt proteins in different treatments, 5–6 specimens of each of the host larvae, parasitoid larvae, cocoons, or freshly emerged adults from three replications in a treatment were collected and crushed together to detect the Bt proteins in the insect body using a double sandwich enzyme-linked immunosorbent assay (ELISA) kit (EnviroLogic Inc., Portland, ME, USA). The *C. chlorideae* larvae were collected from the live *H. armigera* larvae. The *H. armigera* larvae showing symptoms of parasitization were dissected, and the parasitoid larvae were collected in Eppendorf tubes when they were ready to emerge from the host larvae for pupation. The host/parasitoid samples (whole body) were crushed together as one sample in phosphate-buffered saline (PBS) buffer in the ratio of 1:10 (insect sample:buffer) in Eppendorf tubes with a plastic pestle. The test samples were then centrifuged at 11 269 *g* for 2–3 min, and 100 µl of supernatant was loaded in the test wells of ELISA plate pre-loaded with 100 µl peroxydase enzyme conjugate. The negative and positive controls, and 0.5, 2.5, and 5.0 ppb Bt standards were run along with the test samples for the comparison of ELISA results. The ELISA plate was incubated for 2 h in moist paper towel fitted in a plastic box. After 2 h of incubation, the test wells were thoroughly washed with PBS buffer giving 5–6 flip washings, and keeping the test wells filled with PBS buffer for 1 min at the end. After washing, the test wells were again loaded with 100 µl TMB. The wells showing a deep blue color indicated the presence of the toxin. After 15 min of incubation, 50 µl 3 M sulphuric acid was added, and observations were recorded on an ELISA plate reader at 450-nM wavelength.

Statistical analysis

Data were subject to analysis of variance (ANOVA) using GenStat, version 8 (GenStat, 2006), and the treatment means were compared by least significant differences (LSD) at P = 0.05. The figures presented in the tables are means across replications with F-probability and LSD values.

Results

Intrinsic toxicity of Bt proteins to *Helicoverpa armigera*

The LC_{50} of the Bt proteins to *H. armigera* was estimated to be 1800 ng ml^{-1} diet for Cry1Ac (Figure 1A), and 3000 ng ml^{-1} for Cry1Ab (Figure 1B). The ED_{50} values for Cry1Ac and Cry1Ab were 2 ng ml^{-1} diet (Figure 1C) and 59 ng ml^{-1} (Figure 1D), respectively. The estimated LC_{50} and ED_{50} concentrations of the Cry proteins were used to assess the effects of Bt proteins on survival, development, and fecundity of the parasitoid, *C. chlorideae* through *H. armigera* larvae fed with the intoxicated artificial diet.

Effects of Bt protein-treated *Helicoverpa armigera* larvae on survival and development of *Campoletis chlorideae*

LC_{50} level. There were significant differences (P<0.05) in larval, pupal, and total developmental periods (Table 1), percentage of pupation ($F_{6,12} = 33.48$, P<0.001; Figure 2A), and adult emergence ($F_{6,12} = 15.92$, P<0.001; Figure 2C) of *C. chlorideae*, when developing in *H. armigera* larvae reared on Cry1Ab- (3000 ng ml^{-1}) or Cry1Ac-treated (1800 ng ml^{-1}) artificial diet. Larval period of *C. chlorideae* was prolonged when *H. armigera* larvae were reared on diet with Cry1Ab or Cry1Ac for 24 h. The *H. armigera* larvae fed with artificial diets with Cry1Ab or Cry1Ac for 24 h after parasitization (– +), and before and after (+ +) parasitization, prolonged the larval period of the parasitoid by 1.7–2.4 days. However, there was no significant effect of Cry1Ab or Cry1Ac on the larval period of *C. chlorideae* reared on Bt-treated *H. armigera* for 24 h before parasitization (+ –). Feeding *H. armigera* larvae with diets with Cry1Ab- or Cry1Ac-treated artificial diet after parasitization increased the pupal period significantly compared to the larvae fed with the standard artificial diet.

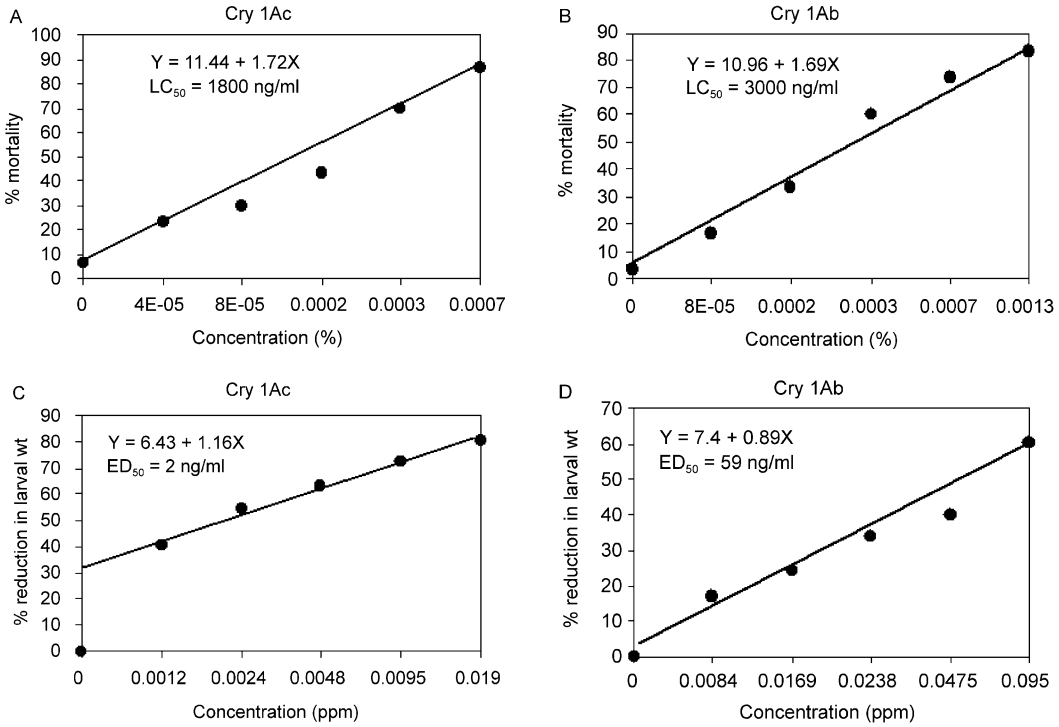

Figure 1 The LC_{50} (effective concentration to kill 50% of the neonate *Helicoverpa armigera* larvae) [(A) Cry1Ac and (B) Cry1Ab] and ED_{50} (effective concentration to cause a 50% reduction in larval weight) [(C) Cry1Ac and (D) Cry1Ab] of *Bacillus thuringiensis* δ-endotoxins against neonate *H. armigera* larvae.

Table 1 Effect of *Bacillus thuringiensis* δ-endotoxin-treated *Helicoverpa armigera* larvae on development, parasitization potential, and sex ratio of the parasitoid, *Campoletis chlorideae*

Treatment[1]	Larval period (days)	Pupal period (days)	Total developmental period (days)	Male weight (mg)	Female weight (mg)	Fecundity/ female	Sex ratio (male:female)
H. armigera fed with artificial diet impregnated with Cry1Ab or Cry1Ac at LC_{50}							
– –Control	8.4	6.2	14.6	2.5	3.2	81.3	1:0.34
– +Cry 1Ab	10.5	6.8	15.7	2.5	3.9	82.2	1:0.51
– +Cry 1Ac	10.1	7.1	17.2	2.2	2.8	80.0	1:0.50
+ –Cry 1Ab	9.1	6.6	17.3	2.4	3.0	60.5	1:0.49
+ –Cry 1Ac	9.1	6.3	15.5	2.4	3.0	69.7	1:0.99
+ +Cry 1Ab	10.8	6.4	16.0	2.3	2.6	63.5	1:0.72
+ +Cry 1Ac	9.6	6.4	17.1	2.2	2.7	70.3	1:0.50
F-probability	0.040	0.007	0.017	0.111	0.518	0.135	0.216
LSD (P = 0.05)	1.48	0.40	1.57	ns	ns	ns	ns
H. armigera fed wtih artificial diet impregnated with Cry1Ab or Cry1Ac at ED_{50}							
– –Control	9.1	6.8	15.9	2.8	3.5	165.7	1:0.22
– +Cry 1Ab	9.0	6.8	16.4	2.6	3.3	129.2	1:0.30
– +Cry 1Ac	8.8	6.5	15.8	2.7	3.2	147.7	1:0.47
+ –Cry 1Ab	9.4	7.0	15.8	2.7	3.3	93.8	1:0.34
+ –Cry 1Ac	9.0	6.8	15.8	2.6	3.2	105.0	1:0.83
+ +Cry 1Ab	9.2	6.6	15.1	2.6	3.1	115.2	1:0.01
+ +Cry 1Ac	8.6	6.4	15.3	2.8	3.3	126.1	1:0.36
F-probability	0.010	0.038	0.005	0.210	0.252	0.005	0.056
LSD (P = 0.05)	0.37	0.32	0.56	ns	ns	32.82	ns

[1]*Helicoverpa armigera* larvae fed with: – –, control diet only; – +, Bt diet after parasitization only; + –, Bt diet before parasitization only; + +, Bt diet before and after parasitization; ns, non-significant.

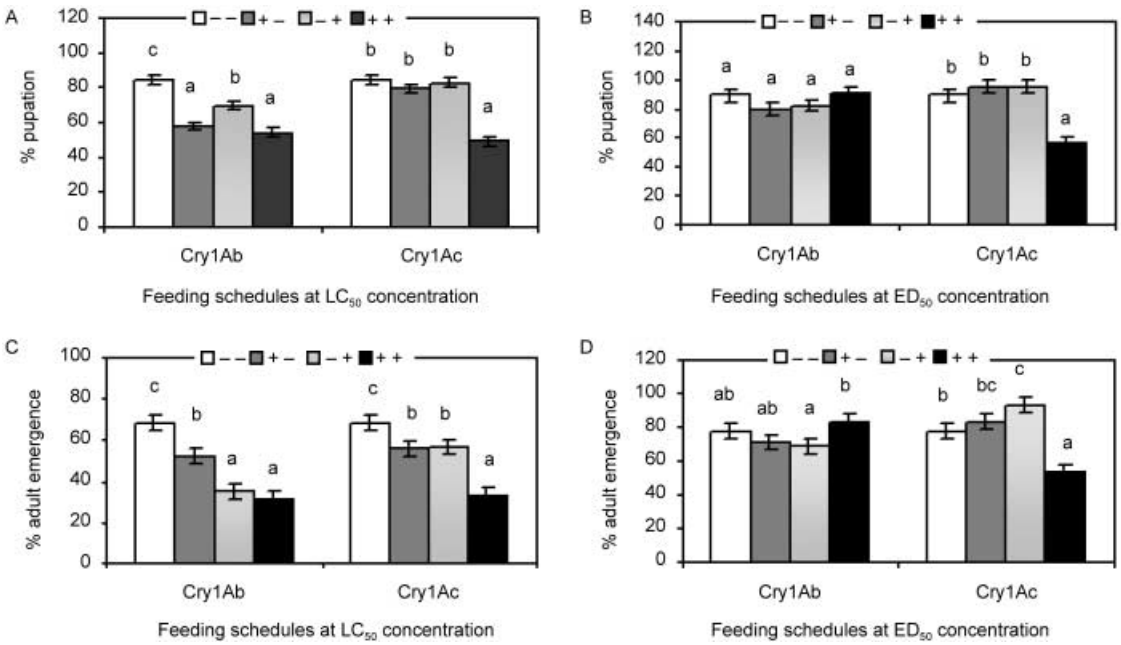

Figure 2 Effect of *Bacillus thuringiensis* δ-endotoxin-intoxicated *Helicoverpa armigera* larvae at LC_{50} and ED_{50} levels on pupation (%) (A and B), and adult emergence (C and D) of the parasitoid, *Campoletis chlorideae* (%) under different feeding treatments: – –, control diet only; – +, Bt diet after parasitization only; + –, Bt diet before parasitization only; and + +, Bt diet before and after parasitization. Bars within a subfigure across feeding treatments of a Bt protein capped with the same letter are statistically non-significant (P>0.05).

Parasitoids reared on *H. armigera* larvae fed for 24 h on Bt diet before and after parasitization showed 30–40% reduction in pupation (Figure 2A), and 25–35% reduction in adult emergence (Figure 2C) over those fed on untreated control diet. Some 17–51% of the *H. armigera* larvae reared for 24 h on Bt-treated diet before and/or after parasitization died before the completion of larval development of *C. chlorideae* (Figure 3A). There were no significant differences in weights of *C. chlorideae* adults (male or female) obtained from Cry1Ab- or Cry1Ac-treated *H. armigera* larvae fed with Bt-treated diet before and/or after parasitization. Although fecundity appeared

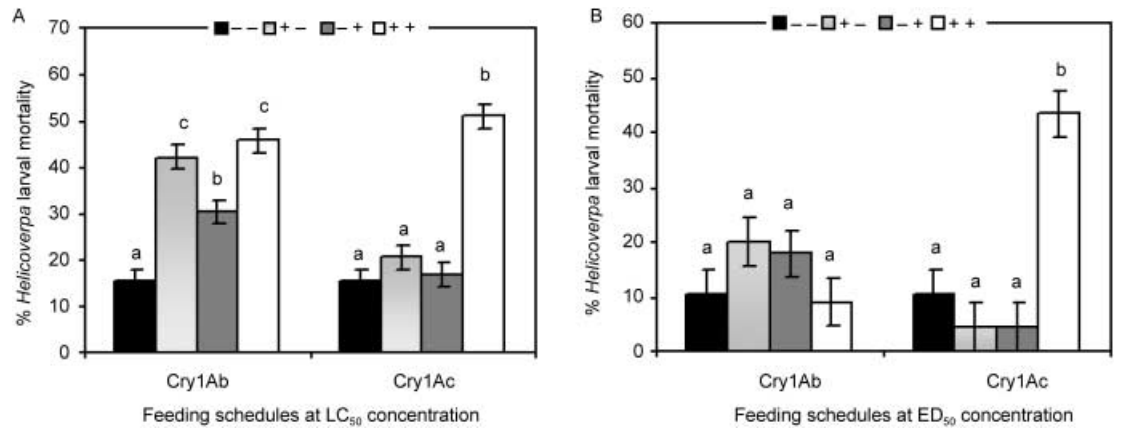

Figure 3 Mortality of *Helicoverpa armigera* larvae before parasitoid emergence at (A) LC_{50} and (B) ED_{50} concentrations of Cry1Ab and Cry1Ac *Bacillus thuringiensis* δ-endotoxins under different feeding treatments: – –, control diet only; – +, Bt diet after parasitization only; + –, Bt diet before parasitization only; + +, Bt diet before and after parasitization. Bars within a subfigure across feeding treatments of a Bt protein capped with same letter are statistically non-significant (P>0.05).

to be reduced in females that had developed in Bt-fed hosts, this difference was not significant (Table 1). There was no change in sex ratio of *C. chlorideae* (Table 1).

ED_{50} level. There were significant differences ($P<0.05$) for larval, pupal, and total developmental periods, fecundity (Table 1), pupation ($F_{6,12} = 10.07$, $P<0.001$; Figure 2B), and adult emergence ($F_{6,12} = 8.33$, $P<0.001$; Figure 2D) of the parasitoid, *C. chlorideae* among different feeding treatments of Cry1Ab or Cry1Ac at the ED_{50} level. However, there was no significant effect of Cry1Ab- or Cry1Ac-treated *H. armigera* larvae on larval and pupal periods of *C. chlorideae*, as compared to those fed with control artificial diet. There was no effect of Bt proteins on pupation of *C. chlorideae* at the ED_{50} level, except when the *H. armigera* larvae were fed with Cry1Ac diet for 24 h (57% pupation on diet with Cry1Ac vs. 89% pupation on control diet) before and after parasitization (Figure 2B). Adult emergence of the parasitoid was 25% lower on *H. armigera* larvae fed with Cry1Ac-treated diet before and after parasitization than of those fed with untreated control diet (Figure 2D). Between 4 and 43% of the *H. armigera* larvae died before the completion of larval development of *C. chlorideae*, when the host larvae were reared on Bt-treated diet for 24 h before and/or after parasitization (Figure 3B). There was no effect of Cry1Ab or Cry1Ac on the weights of male or female adults of *C. chlorideae* (Table 1). Fecundity of the *C. chlorideae* females obtained from *H. armigera* larvae fed with diets with Cry1Ab and Cry1Ac for 24 h (except those on Cry1Ac, – +) was significantly lower (94–129 eggs/female) than those reared on the control diet (166 eggs female^{-1}) (Table 1). There was no effect on sex ratio of *C. chlorideae* adults obtained from *H. armigera* larvae fed with Bt protein-intoxicated diets (Table 1).

Detection of Bt proteins

The ELISA test indicated the presence of Bt proteins in the *H. armigera* larvae, while no Bt proteins were detected in the larvae, cocoons, and adults of *C. chlorideae* obtained from *H. armigera* larvae fed with the Cry1Ab or Cry1Ac diets at LC_{50} or ED_{50} levels.

Discussion

The impact of Bt-transgenic crop plants on biological control organisms has received considerable attention (O'Callaghan et al., 2005; Romeis et al., 2006; Sharma et al., 2007). It is generally recognized that host-plant quality influences the feeding, growth, and development of phytophagous insects, which can have profound effects on the tritrophic interactions between plants, herbivores, and their natural enemies (Price et al., 1980; Price, 1986; Murugan & George, 1992; Jeyabalan & Murugan, 1996; Dhillon & Sharma, 2007). Parasitic wasps are particularly sensitive to changes in the nutritional quality of their hosts, as host–parasitoid relationships are usually quite intricate (Godfray, 1994). It is thus not surprising that the parasitoid activity is affected when their hosts are affected by the Bt protein. This can be due to a poor host quality or due to reduction in host population (which is the goal of the Bt crops). Sublethal effects of Bt proteins on the host larvae (sick host) may reduce their nutritional quality for the parasitoid, and poor nutritional quality of the host results in detrimental effects on the development and survival of the natural enemies (Nordlund et al., 1988; Murugan et al., 2000). However, these prey quality-mediated effects are less likely for predators (Romeis et al., 2006).

The microbial products from *B. thuringiensis* have been reported to be safer to non-target organisms (Croft, 1990; Entwistle et al., 1993), and are generally regarded as compatible with biological control agents (Wright & Verkerk, 1995). The Bt protein Cry1Ac has been reported to be 1.93-fold more effective against *H. armigera* than Cry1Ab at the LC_{50} level (Mane & Nikat, 2003). In the current studies, it was found to be 1.67-fold more toxic than Cry1Ab. However, the *H. armigera* reared on Cry1Ac-treated diet at ED_{50} had as high a mortality (43.3%) as at LC_{50} (51.1%). This might be because of variability in levels of sensitivity in the group of test insects to the Bt protein. The Bt proteins fed to *H. armigera* larvae for 24 h before and after parasitization resulted in more detrimental effects on the survival and development of the parasitoid, *C. chlorideae* as compared to those fed with Bt-treated diet before or after parasitization, except in a few cases. However, there were no significant differences in survival of *C. chlorideae* when reared on *H. armigera* fed with Bt-treated artificial diet for 24 h before or after parasitization, except for the larvae fed with diet with Cry1Ab at the LC_{50} level. The exposure of host larvae to Bt proteins before and after parasitization by parasitoids is more realistic under field conditions, where the host larvae will have continuous exposure to the expressed Bt toxins and deliver continuous indirect effects. Such negative indirect effects of continuous exposure of host lepidopteran larvae to Bt-transgenic cotton under field conditions have earlier been reported on larval parasitoids, *Cotesia marginiventris* (Cresson), *Copidosoma floridanum* (Ashmead) (Baur & Boethel, 2003), and *C. chlorideae* (Liu et al., 2005). Zhang et al. (2006) observed low effects of Bt on *C. chlorideae* attacking Cry1Ac-resistant *H. armigera* larvae treated with the HD-73 strain of Bt containing only 44% of Cry1Ac. This might have been because of the sublethal dose of the Bt toxin on which more Bt-resistant *H. armigera* larvae survived, resulting in lower effects on the survival of the parasitoid.

In the present study, Bt-fed *H. armigera* affected the pupation and adult emergence of *C. chlorideae*. The adverse effects on *C. chlorideae* were mainly because of early mortality of Bt-fed *H. armigera* larvae within 5–6 days, while the parasitoid requires 8–9 days to complete post-embryonic development (Sharma et al., 2007). These were largely indirect effects (host quality mediated) involving Bt-sensitive lepidopteran larvae. Predators, on the other hand, do not show such a strong response to altered prey quality. Nevertheless, all cases in which detrimental effects were observed for predators in laboratory studies involved the use of sensitive prey organisms. *Chrysoperla carnea* (Stephens) is an excellent example of such indirect effects (Romeis et al., 2006).

Meissle et al. (2004) detected Cry1Ab toxin in cocoons of the parasitoid *Campoletis sonorensis* (Cameron), when reared on *Spodoptera littoralis* (Boisd.) larvae fed on Bt-transgenic maize. The result indicates that the parasitoids had ingested the Cry toxin when feeding inside the host larvae. In contrast, Cry1Ab was not detected in the cocoons of *C. marginiventris* when reared on Bt-transgenic maize-fed *S. littoralis*, but was present in trace amounts in the empty cocoons (Vojtech et al., 2005). Similarly, we did not detect Cry proteins in larvae of *C. chlorideae* that had developed within Bt-fed host larvae. Although *H. armigera* larvae fed with Bt proteins in the present studies showed some adverse effects on *C. chlorideae*, these effects were indirect (host mediated), as there was no Bt protein detected in any of the life stages of the parasitoid. These findings will be useful to develop appropriate strategies for assessing the risks of transgenic crops to the parasitoids associated with insects feeding on Bt-transgenic crops, and safe deployment of transgenic crops for sustainable crop production. Furthermore, studies suggest that the reduction in *C. chlorideae* population on Bt-susceptible *H. armigera* larvae on the Bt-transgenic crops would be density dependent (Dhillon & Sharma, 2007), and the parasitoid might have some level of survival on the Bt-resistant host insects to help manage the populations of Bt-resistant *H. armigera* in the transgenic crops.

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