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Influence of transgenic cottons with *Bacillus thuringiensis cry1Ac* gene on the natural enemies of *Helicoverpa armigera*

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Abstract. Transgenic cotton has been released for cultivation in several parts of the world to increase crop productivity. However, concerns have been raised regarding the possible undesirable effects of genetically modified crops on non-target organisms in the eco-system. Therefore, we studied the effects of transgenic cottons with *cry1Ac* gene from *Bacillus thuringiensis* Berliner (*Bt*) on the natural enemies of cotton bollworm/legume pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) under field and laboratory conditions. There was no apparent effect of transgenic cotton on the relative abundance of predatory spiders (*Clubiona* sp. and *Neoscona* sp.), coccinellid (*Cheilomenes sexmaculatus* Fab.), and the chrysopid (*Chrysoperla carnea* Stephens). However, the abundance of spiders, coccinellids, and chrysopids was quite low in insecticide protected plots towards end of the cropping season. There was a significant reduction in cocoon formation and adult emergence of the ichneumonid parasitoid, *Campoletis chlorideae* Uchnida reared on *H. armigera* larvae fed on the leaves of transgenic cottons before and after parasitization. However, no *Bt* toxins were detected in *H. armigera* larvae and the parasitoid cocoons with enzyme linked immunosorbent assay. Reduction in cocoon formation was because of early mortality of the *H. armigera* larvae due to *Bt* toxins in the leaves of transgenic cotton. There was a slight reduction in adult weight and fecundity, and prolongation of the larval period when the parasitoid was raised on *H. armigera* larvae fed on the leaves of transgenic cotton before and after parasitization. Survival and development of *C. chlorideae* was also poor when *H. armigera* larvae were fed on the leaves of cotton hybrid Mech 184. The adverse effects of transgenic cotton on survival and development of *C. chlorideae* were largely due to early mortality, and possibly poor nutritional quality of *H. armigera* larvae due to toxic effects of the transgene.

Key words: *Campoletis*, *Helicoverpa*, Lepidoptera, Noctuidae, non-target effects, parasitoids, predators, transgenic cotton

Introduction

Insect-resistant transgenic plants with *Bacillus thuringiensis* (*Bt*) toxins genes have been developed in several crops (Hilder and Boulter, 1999; Sharma et al., 2000, 2004), of which transgenic cotton with *Bt* toxin genes has now been deployed for controlling bollworms in USA, Australia, China, South Africa, and India (James, 2003). While considerable information has been generated on the relative efficacy of transgenic crops against the target and non-target insect pests in USA, Australia, and China (Wilson et al., 1992; Benedict et al., 1996; Ni et al., 1996; Guo et al., 1999; Cui and Xia, 1999; Greenplate, 1999; Fitt, 2003), there is a limited information on these aspects in the tropics, where the transgenic crops have been deregulated for cultivation only recently (Qaim and Zilberman, 2003).

One of the major concerns of deployment of transgenic crops is their effect on the non-target organisms. Continuous availability of *Bt* proteins in the crop plants in the modified form and mode of release prohibits a simple deduction of the safety of the transgenic plants based on the past safety record of *Bt* insecticide sprays (Sharma and Ortiz, 2000). Several workers have studied the effects of transgene products and transgenic crops on the relative abundance of natural enemies under field and laboratory conditions (Hoffmann et al., 1992; Sims, 1995; Flint et al., 1995; Luttrell et al., 1995; Orr and Landis, 1997; Wang and Xia, 1997; Shelton et al., 2002). Liu et al. (2003) observed that presence of the *cry1Ab* gene had no marked effect on predation by the wolf spider, *Pirata subpiraticus* (Bös. st Str.) on the brown planthopper, *Nilaparvata lugens* (Stal.) and the rice leaffolder, *Cnaphalocrocis medinalis* (Guen.). *Bt*-cotton has been reported to increase the diversity of arthropod communities and pest sub-communities. However, it decreased the diversity of natural enemy sub-communities (Men et al., 2003). It is quite difficult to have a clear understanding of the effects of transgenic plants on the abundance of generalist predators, which feed on several alternate prey, and in many cases, the prey may or may not imbibe the *Bt* toxins from the plants. The populations of generalist predators also fluctuate in cycles of several generations. Transgenic plants have been reported to act synergistically in combination with *Campoletis sonorensis* (Cameron) and decrease the survival of *Heliothis virescens* (F.) larvae beyond the level expected for an additive interaction. There are no major effects of transgenic plants on the activity of *Cardiochiles nigriceps* Viereck under field conditions (Johnson, 1997; Johnson et al., 1997). Lu et al.

(2004) reported that cocoon formation and weight of *Microplitis mediator* Haliday and *Campoletis chlorideae* Uchida reared on *H. armigera* (Hubner) fed on transgenic cotton declined greatly. Cocoon formation and cocoon weight decreased by 26.1 and 17.9%, and 1.0 and 5.1 mg, respectively. There is little information on the possible effects of transgenic crops on the generalist predators and host specific parasitoids in the tropics. Therefore, the present studies were undertaken to understand the effects of transgenic cotton on the generalist predators of *H. armigera* under field conditions, and that of the host specific parasitoid, *C. chlorideae* under laboratory conditions.

Materials and methods

Cotton cultivars comprising of *Gossypium hirsutum* transgenic and non-transgenic hybrids (Mech 12, Mech 162, and Mech 184 supplied by the Mahyco Seeds Ltd.), two non-transgenic commercial varieties each of *G. hirsutum* (LK 861 and L 604) and *G. arboreum* (Aravinda and MDL 2450) were grown under field conditions on deep black soils (Vertisols) under protected [need based application of insecticides for the control of leafhopper, *Amracsa biguttula biguttula* Ishida, and cotton bollworms, *H. armigera*, *Earias vittella* (Fab.), and *Pectinophora gossypiella* (Saunders) as indicated in Table 1] and unprotected (no insecticide application) conditions during the 2002–2004 rainy seasons. The crop was raised under rainfed conditions during the rainy season (June–Dec). Normal agronomic practices were followed for raising the crop (basal fertilizer N:P:K::100:40:60 kg ha^{-1}). Carbofuran 3G granules were applied around the seedlings (3 g $plant^{-1}$) at 10 days after seedling emergence to protect the crop from damage by the jassid, *A biguttula biguttula* during the 2002 cropping season, while monocrotophos sprays were applied during the 2003 and 2004 cropping seasons at 15 days after seedling emergence.

The test cultivars were planted in a randomized complete block design, and there were three replications under protected and unprotected conditions. The protected and unprotected plots were separated from each other by 4 m and considered as independent experiments. The seeds were planted on ridges 75 cm apart, and spaced at 50 cm. For each plot, there were four rows, 4 m long. No insecticide sprays were applied in the unprotected plots. Amount of spray applied was 250–300 l ha^{-1}, depending on the growth of the crop. Data were recorded on egg and larval parasitoids from samples collected in the field. The numbers of generalist predators such

Table 1. Spray schedule for insect pest management on transgenic and non-transgenic cotton cultivars across seasons (ICRISAT, Patancheru 2002–2004 rainy seasons)

Days after seedling emergence	Target insects	2002	2003	2004
< 60	Jassids, bollworms	Monocrotophos 36%SL	Monocrotophos 36%SL, methomyl 40%SP	Imidacloprid 17.5%SL, methomyl 40%SP
61–80	Jassids, bollworms	Methomyl 40%SP, monocrotophos 36%SL	Methomyl 40%SP	Monocrotophos 36%SL
81–120	Bollworms, aphids and whiteflies	Cypermethrin 25%EC, endosulfan 35%EC, methomyl 40%SP	Endosulfan 35%EC, cypermethrin 25%EC, methomyl 40%SP	Methomyl 40%SP, cypermethrin 25%EC
> 120	Bollworms and aphids	Cypermethrin 25%EC, monocrotophos 36%SL	Cypermethrin 25%EC, monocrotophos 36%SL, methomyl 40%SP	Monocrotophos 36%SL, methomyl 40%SP

The rates of insecticide application were: 1,000 g *ai* ha^{-1} for monocrotophos, 500 g *ai* ha^{-1} for methomyl, 40 g *ai* ha^{-1} for cypermethrin, and 700 g *ai* ha^{-1} for endosulfan.

as coccinellids – *Cheilomenes sexmaculatus* F., chrysopids – *Chrysoperla carnea* (Stephen), and spiders – *Clubiona* sp. and *Neoscona* sp. were recorded at different intervals in the field. Experiments were also conducted on the survival and development of larval parasitoid, *C. chlorideae* on *H. armigera* larvae fed on the leaves of transgenic and non-transgenic cottons before and/or after parasitization under laboratory conditions.

Effect of transgenic cottons on relative abundance of natural enemies

Data on the relative abundance of natural enemies was recorded both under protected and unprotected conditions. Twenty-five eggs and 25 larvae of *H. Armigera* were collected from each plot at the 50% fruiting stage of the crop. The eggs and larvae were placed individually in glass vials (15 ml capacity), brought to the laboratory, and kept under ambient conditions in the laboratory. Larvae were reared on artificial diet (Armes et al., 1992) till adult emergence. Data were also recorded on the numbers of spiders (*Clubiona* sp. and *Neoscona* sp.) (at 55, 60 and 140, and 60 and 130 days after crop emergence during the 2002, 2003, and 2004 cropping seasons, respectively), coccinellid, *C. sexmaculatus* (at 55, 30, 60 and 140, and 60 and 130 days after crop emergence during the 2002, 2003, and 2004 cropping seasons, respectively), and the chrysopid, *C. carnea* (at 60 and 140, and 60 and 130 days after crop emergence during the 2003 and 2004 cropping seasons, respectively). The predator population was recorded on five plants tagged at random in the center of each plot.

Effect of transgenic plants on the survival and development of the parasitoid, Campoletis chlorideae

To assess the adverse effects of transgenic plants on the host specific parasitoid of *H. armigera*, we reared the *H. armigera* larvae on the leaves of transgenic plants before and/or after parasitization by the females of the parasitoid wasp, *C. chlorideae*. The treatments consisted of feeding *H. armigera* larvae on leaves of transgenic hybrids (Mech 12, Mech 162, and Mech 184) for 2 or 5 days before parasitization, and then on the leaves of transgenic (+ +) or non-transgenic hybrids (+ −) till cocoon formation. In another set of treatments, the larvae were first fed on the leaves of non-transgenic hybrids (Mech 12, Mech 162, and Mech 184) for 2 or 5 days before parasitization, and then on the leaves of transgenic (− +) or non-transgenic (− −)

hybrids. Five-day-old larvae were exposed individually to the freshly mated females of *C. chlorideae* for oviposition individually in a 20 ml glass vial. After parasitization (in nearly 1–2 min), the larvae were transferred to the leaves of transgenic or non-transgenic hybrids till cocoon formation or mortality of *H. armigera* larvae. The leaves of transgenic or non-transgenic hybrids (as per different treatment combinations) were held in 3% agar–agar medium in 250 ml plastic cups (Sharma et al., 2005). This system maintained the leaves in a turgid condition for 1 week. There were three replications for each treatment, and each replication had 20 larvae in a completely randomized design. The leaves were changed every 5 days. The *C. chlorideae* females were used to parasitize *H. armigera* larvae for three consecutive days. Data were recorded on the number of parasitoid cocoons formed and the adults emerged, duration of egg + larval period (since egg hatching took place inside the *H. armigera* larvae), pupal period, and the weight of the adults that emerged from *H. armigera* larvae fed on the leaves of transgenic or non-transgenic cotton before and/or after parasitization. Observations were also recorded on the sex ratio (males:females) of the progeny, and the fecundity (number of progeny produced per female). For fecundity studies, 5-day-old *H. armigera* larvae were exposed to the mated females of *C. chlorideae* for oviposition (nearly 20 larvae per day) till the death of the parasitoid females. The number of progeny produced per female was taken as a measure of fecundity.

The amounts of Cry1Ac toxin in the leaves of transgenic cotton hybrids, on which the *H. armigera* larvae were fed before and/or after parasitization, were estimated by enzyme linked immunosorbent assay (ELISA) using a commercially available kit (EnviroLogic, Inc., Portland, USA). Leaf samples (100 mg) were taken from the same plants from which the leaves were used for feeding the *H. armigera* larvae, and placed in 5 ml stoppered vials in liquid nitrogen at -80 °C. For estimating the *Bt* toxins, the samples were taken out from liquid nitrogen, and allowed to equilibrate at the room temperature. Twenty milligrams of leaf tissue was taken in an eppendorf tube and ground for 20–30 s. Extraction/dilution buffer (0.5 ml, $1\times$) was added to each eppendorf tube and grinding was repeated. The solids were allowed to settle at the bottom of the vial for a few minutes. The sample extract was diluted to 1:2.78, 1:5.56, and 1:11.11 with Cry1Ac extraction buffer. A standard curve with Cry1Ab (supplied with the kit) was prepared to estimate the amounts of *Bt* toxins in leaves, *H. armigera* larvae, and the parasitoid cocoons. The amounts of *cry*

toxins in the leaf tissue were converted into Cry1Ac equivalents by using an appropriate conversion factor, and expressed as ppm.

To determine whether the adverse effects of transgenic cotton plants on parasitoid were due to *Bt* toxins ingested by the *H. armigera* larvae, three larvae (after feeding on the transgenic or non-transgenic leaves before parasitization), three parasitoid cocoons, and remnants of three larvae after cocoon formation were placed in 5 ml vials, which were caped tightly, and placed in liquid nitrogen at − 80 °C. For estimating the *Bt* toxins, the samples were taken out from liquid nitrogen, allowed to equilibrate at the room temperature, weighed, macerated in extraction buffer, and subjected to ELISA test as described above.

Statistical analysis

The data were subjected to analysis of variance using randomized block design for each date of observation for the respective predator species. The significance of differences between the treatments was judged by *F*-test at $p \leq 0.05$, while the significance of differences between the treatment means was determined by least significant difference (LSD) at $p \leq 0.05$.

Results

Effect of transgenic cottons and protection regimes on relative abundance of natural enemies under field conditions

No egg and larval parasitoids were recorded in the egg and larval samples collected from the field. The results indicated complete absence of parasitoids from the cotton eco-system at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) farm. There were no differences in the numbers of spiders on the *Bt* transgenic and non-transgenic cottons under unprotected or protected conditions at 55–60 days after crop emergence (Table 2). However, there was a substantial reduction in the spider numbers at 140–130 days after crop emergence in plots under insecticide protection during the 2003 and 2004 cropping seasons.

There were no significant differences in the relative abundance of the coccinellid, *C. sexmaculatus* within a protection regime or seasons (Table 3). Coccinellid numbers were greater on the transgenic than on the non-transgenic hybrid under unprotected conditions during the

Table 2. Relative abundance of predatory spiders (*Clubiona* sp. and *Neoscona* sp.) on *Bt* transgenic and non-transgenic commercial cultivars of *Gossypium hirsutum* and *Gossypium arboreum* cottons (ICRISAT, Patancheru, 2002–2004)

Cultivar	No. of spiders per 5 plants									
	2002		2003				2004			
	55 DAE		60 DAE		140 DAE		60 DAE		130 DAE	
	UP	CP	UP	CP	UP	CP	UP	CP	UP	CP
Gossypium arboreum varieties										
Aravinda	10.0	16.7	6.4	1.7	1.7	0.0	0.7	1.3	1.7	0.0
MDL 2450	15.0	8.4	5.0	2.7	1.7	0.0	0.3	1.0	9.0	0.0
Gossypium hirsutum varieties										
L 604	5.0	21.7	3.4	3.4	1.7	0.0	1.3	1.7	7.7	2.3
LK 861	15.0	13.4	2.5	2.0	0.6	1.1	0.7	1.7	7.0	2.3
Transgenic hybrids										
Mech 12	–	–	4.7	2.7	1.7	0.0	1.3	2.3	5.0	1.3
Mech 162	23.4	16.7	5.0	2.7	2.2	0.0	0.7	2.7	5.7	0.7
Mech 184	–	–	6.7	2.4	1.1	0.0	2.7	2.7	0.7	0.3
Non-transgenic hybrids										
Mech 12	–	–	3.0	3.7	2.2	0.6	1.7	3.0	6.0	2.3
Mech 162	11.7	20.0	4.7	3.4	2.8	0.0	1.0	2.0	2.3	1.3
Mech 184	–	–	7.0	4.4	2.2	0.0	4.3	6.3	1.7	0.0
SE ±	5.1	7.6	1.4	0.9	1	0.3	0.9	1.55	2.33	1.07
LSD (p 0.05)	16(NS)	23.9(NS)	4.2(NS)	2.5(NS)	2.9(NS)	0.7(NS)	2.68(NS)	4.62(NS)	6.93(NS)	3.17(NS)
Fp (df = 9)	0.284	0.696	0.343	0.53	0.912	0.053	0.139	0.521	0.208	0.555

DAE, Days after seedling emergence; UP, Unprotected; CP, Completely protected; –, Not tested; NS, Non-significant; *Fp*, Probability of *F*-test; df, Degrees of freedom.

Table 3. Relative abundance of coccinellids (*Cheilomenes sexmaculatus*) on *Bt* transgenic and non-transgenic commercial cultivars of *Gossypium hirsutum* and *Gossypium arboreum* cottons (ICRISAT, Patancheru, 2002–2004)

Cultivar	No. of coccinellids per 5 plants													
	2002		2003						2004					
	55 DAE		30 DAE		60 DAE		140 DAE		30 DAE		60 DAE		130 DAE	
	UP	CP	UP	CP	UP	CP	UP	CP	UP	CP	UP	CP	UP	CP
Gossypium arboreum varieties														
Aravinda	21.7	16.7	1.0	1.0	1.3	0.0	0.6	0.0	2.3	3.7	3.7	0.7	1.0	0.0
MDL 2450	3.4	10.0	1.0	2.3	0.0	0.0	1.7	0.0	2.7	2.3	2.3	0.0	1.0	0.0
Gossypium hirsutum varieties														
L 604	18.4	16.7	0.7	4.0	0.3	0.7	0.0	0.0	2.7	3.3	0.7	0.0	0.3	0.0
LK 861	11.7	16.7	2.3	3.3	0.3	0.0	0.0	0.0	2.7	1.0	1.0	1.3	0.3	0.0
Transgenic hybrids														
Mech 12	–	–	0.3	1.3	0.3	0.0	0.6	0.0	7.7	1.0	0.3	1.7	1.7	0.0
Mech 162	35.0	10.0	1.0	1.7	0.3	0.3	0.6	0.0	2.7	3.0	0.3	0.3	1.0	0.0
Mech 184	–	–	0.7	4.3	1.0	0.3	0.0	0.0	3.3	4.0	0.3	1.0	0.0	0.0
Non-transgenic hybrids														
Mech 12	–	–	1.0	1.7	1.0	0.0	3.3	0.0	5.0	3.3	0.7	0.3	0.0	0.3
Mech 162	13.4	1.7	0.0	1.3	0.0	0.3	0.6	0.0	5.0	3.0	0.7	0.0	1.0	0.3
Mech 184	–	–	1.3	2.0	0.3	0.3	0.6	0.0	4.0	3.0	1.0	1.0	1.0	0.0
SE ±	6.0	6.0	0.6	0.9	0.6	0.2	0.9	–	1.3	1.38	0.96	0.54	0.67	0.14
LSD (p 0.05)	18.9(NS)	18.9(NS)	1.9(NS)	2.6(NS)	1.7(NS)	0.7(NS)	2.7(NS)	–	3.86(NS)	4.11(NS)	2.85(NS)	1.61(NS)	2.0(NS)	0.42(NS)
Fp (df = 9)	0.069	0.465	0.481	0.124	0.778	0.519	0.298	–	0.17	0.815	0.321	0.345	0.748	0.474

DAE, Days after seedling emergence; UP, Unprotected; CP, Completely protected; –, Not tested; NS, Non-significant; *Fp*, Probability of *F*-test; df, Degrees of freedom.

2002 cropping season. High numbers of the coccinellids were also recorded on the non-transgenic commercial varieties; Aravinda, L 604, and LK 861 at 55 days after crop emergence. During the 2003 and 2004 cropping seasons, there were no differences in coccinellid numbers between the cultivars tested. The coccinellids were nearly absent in the protected plots at 130–140 days after crop emergence, indicating that insecticide application has a deleterious effect on the abundance of predatory coccinellids.

There were no significant differences in the numbers of chrysopid larvae among the cultivars tested within a protection regime, except in

Table 4. Relative abundance of chrysopids (*Chrysoperla carnea*) on *Bt* transgenic and non-transgenic commercial cultivars of *Gossypium hirsutum* and *Gossypium arboreum* cottons (ICRISAT, Patancheru, 2003/2004)

Cultivar	No. of chrysopids per 5 plants							
	2003				2004			
	60 DAE		140 DAE		60 DAE		130 DAE	
	UP	CP	UP	CP	UP	CP	UP	CP
Gossypium arboreum varieties								
Aravinda	5.0	1.7	14.5	1.1	0.7	1.3	1.7	0.0
MDL 2450	1.7	4.7	12.2	4.5	0.3	1.0	9.0	0.0
Gossypium hirsutum varieties								
L 604	6.0	10.7	4.5	2.8	1.3	1.7	7.7	2.3
LK 861	3.1	3.7	8.9	1.1	0.7	1.7	7.0	2.3
Transgenic hybrids								
Mech 12	5.0	9.4	3.4	7.8	1.3	2.3	5.0	1.3
Mech 162	4.1	13.4	5.0	3.9	0.7	2.7	5.7	0.7
Mech 184	9.7	12.0	3.4	2.2	2.7	2.7	0.7	0.3
Non-transgenic hybrids								
Mech 12	3.7	7.0	7.2	0.0	1.7	3.0	6.0	2.3
Mech 162	5.4	5.4	8.9	3.9	1.0	2.0	2.3	1.3
Mech 184	2.4	11.0	14.5	3.4	4.3	6.3	1.7	0.0
SE±	1.8	3.1	4.1	2.6	0.9	1.55	2.33	1.07
LSD (*p* 0.05)	5.4(NS)	9.1(NS)	12.1(NS)	7.7(NS)	2.68(NS)	4.62(NS)	6.93(NS)	3.17(NS)
Fp (df = 9)	0.214	0.135	0.027	0.689	0.139	0.521	0.208	0.555

DAE, Days after seedling emergence; UP, Unprotected; CP, Completely protected; NS, Non-significant; *Fp*, Probability of *F*-test; df, Degrees of freedom.

unprotected plots at 140 days after crop emergence (Table 4). The numbers of chrysopid, *C. carnea* larvae were lower in plots receiving complete protection than in the unprotected plots at 130–140 days after crop emergence (except in Mech 12 during 2003 cropping season). The numbers of chrysopid larvae were greater on the varieties Aravinda, L 604, and Mech 184 both under protected and unprotected conditions, although there were a few exceptions.

Effect of transgenic cottons on the survival and development of Campoletis chlorideae

Post-embryonic development

The differences in egg + larval periods of *C. chlorideae* were not large when the *H. armigera* larvae were fed for 2 days on the leaves of non-transgenic hybrids (9.13–10.24 days) or on the leaves of transgenic hybrids before or after parasitization (9.40–12.94 days) (Table 5). However, there was some prolongation of the egg + larval period when the parasitoid was reared on *H. armigera* larvae fed on the leaves of transgenic hybrids before and after parasitization (12.53–13.50 days). Prolongation of egg + larval period was also observed when the parasitoid was reared on *H. armigera* larvae fed on the leaves of transgenic or non-transgenic versions of Mech 184. The pupal period ranged from 6.08–7.62, and 5.48–6.89 days, when the parasitoid was reared on *H. armigera* larvae fed on transgenic and/or non-transgenic cottons for 2 and 5 days, respectively. Total development period of *C. chlorideae* was marginally prolonged when the *H. armigera* larvae were fed on the leaves of transgenic hybrids before and after parasitization (18.61–20.15 days) as compared to the larvae fed on the leaves of non-transgenic hybrids (15.70–16.92 days). Similar trends in prolongation of post-embryonic development period, though less pronounced, were also observed when the *H. armigera* larvae were fed on the leaves of respective transgenic or non-transgenic hybrids for 5 days.

Pupation and adult emergence

Cocoon formation was significantly lower when the parasitoid was reared on *H. armigera* larvae fed on leaves of the transgenic plants before and after parasitization (4.44–22.22%) or on the leaves of transgenic plants after parasitization (6.67–28.89%) as compared to the parasitoids raised on *H. armigera* larvae fed on the leaves of non-transgenic plants before and after parasitization (55.56–95.56%) (Table 6). Cocoon formation was also low when the *H. armigera*

Table 5. Post-embryonic development period of the parasitoid, *Campoletis chlorideae* raised on *Helicoverpa armigera* larvae reared on leaves of transgenic cotton plants expressing *Bacillus thuringiensis* toxin Cry1Ac for different intervals (ICRISAT, Patancheru, 2003)

Cultivar/treatment	Longevity (days)[a]		Egg + larval development period (days)		Pupal period (days)		Post-embryonic development period (days)	
	2 days[b]	5 days[b]	2 days[b]	5 days[b]	2 days[b]	5 days[b]	2 days[b]	5 days[b]
Mech 12 − −	8.00cd	7.17e	9.13a	9.57ab	7.56de	6.13b	16.69ab	15.70
Mech 162 − −	7.00abc	6.87de	9.49a	9.42a	6.21ab	6.14b	15.70a	15.56
Mech 184 − −	8.91d	6.70cde	10.24ab	9.31a	6.68abc	6.31b	16.92ab	15.62
Mech 12 + −	6.92abc	6.94de	9.40a	10.61c	7.62e	5.93ab	17.02ab	16.54
Mech 162 + −	7.15abc	6.47bcde	10.16ab	9.63abc	6.13ab	6.28b	16.29ab	15.91
Mech 184 + −	7.61bc	6.30bcde	10.62abc	10.64c	6.69abc	6.83cd	17.31abc	17.47
Mech 12 − +	6.59ab	6.35bcde	12.27cde	11.67d	7.55de	5.98ab	19.82d	17.65
Mech 162 − +	6.85abc	6.08bcd	11.43bcd	9.02a	6.75bc	6.89d	18.18abcd	15.91
Mech 184 − +	7.04abc	6.09bcd	12.94de	10.39bc	6.61abc	6.33bc	19.58cd	16.72
Mech 12 + +	6.06a	4.10a	12.58de	11.58d	6.93cd	5.48a	19.51cd	17.06
Mech 162 + +	6.73ab	5.68bc	13.50e	9.94abc	6.65abc	5.99ab	20.15d	15.93
Mech 184 + +	6.54ab	5.67b	12.53cde	12.05d	6.08a	–	18.61bcd	–
SE ±	0.42	0.35	0.65	0.31	0.21	0.17	0.84	1.08
LSD (p 0.05)	1.23	1.02	1.92	0.92	0.63	0.51	2.48	3.178(NS)
Fp (df)	0.011(11)	< 0.001(11)	< 0.001(11)	< 0.001(11)	< 0.001(11)	< 0.001(11)	< 0.001(11)	< 0.001(11)

[a]Longevity of *Helicoverpa armigera* larvae excluding those from which the parasitoid cocoons emerged.
[b]Duration of feeding the larvae on the respective cultivars before parasitization.
− −, *Helicoverpa armigera* larvae fed on control non-transgenic cotton leaves before and after parasitization; + −, *Helicoverpa armigera* larvae fed on leaves of transgenic cotton plants before parasitization; − +, *Helicoverpa armigera* larvae fed on leaves of transgenic cotton plants after parasitization till cocoon formation; + +, *Helicoverpa armigera* larvae fed on leaves from transgenic cotton plants before and after parasitization; *Fp*, Probability of *F*-test; df, Degrees of freedom; NS, Non-significant.
Figures followed by the same letter within a column are not significantly different at $p \leq 0.05$.

Table 6. Survival of the parasitoid, *Campoletis chlorideae*, raised on *Helicoverpa armigera* larvae fed on transgenic cotton plants expressing *Bacillus thuringiensis* toxin Cry1Ac for different intervals (ICRISAT, Patancheru, 2002–2004)

Cultivar/treatment	Cocoon formation (%)		Adult emergence (%)		Adult weight (mg)		Fecundity (No. of cocoons per female)		Sex ratio (Males:Females)	
	2 days[a]	5 days[a]	2 days[a]	5 days[a]	2 days[a]	5 days[a]	2 days[a]	5 days[a]	2 days[a]	5 days[a]
Mech 12 − −	95.56e	73.33e	91.11f	66.67e	2.34f	2.04d	184	123	1:0.55	1:0.39
Mech 162 − −	95.56e	71.11de	93.33f	62.22de	2.23ef	1.90cd	71	74	1:0.96	1:0.68
Mech 184 − −	75.56d	55.56d	73.33e	42.22c	1.88de	1.95d	–	–	–	–
Mech 12 + −	71.11d	57.78de	66.67de	46.67cd	2.02ef	1.71bcd	84	85	1:0.46	1:0.84
Mech 162 + −	71.11d	60.00de	66.67de	40.00c	1.88de	1.73bcd	–	71	–	1:0.65
Mech 184 + −	68.89d	31.11c	57.78d	20.00b	1.59cd	1.69bc	–	69	–	1:0.70
Mech 12 − +	13.33ab	15.56abc	21.48c	4.44ab	0.98a	1.92d	–	62	–	1:0.89
Mech 162 − +	28.89c	24.44bc	22.22c	15.56ab	1.43bc	1.81bcd	66	70	1:0.44	1:0.97
Mech 184 − +	6.67a	17.78abc	18.92bc	8.89ab	1.07ab	1.50abc	–	73	–	1:0.30
Mech 12 + +	13.33ab	13.33ab	6.67ab	4.44ab	0.98a	1.17a	–	66	–	1:0.45
Mech 162 + +	22.22bc	4.44a	15.56abc	5.93ab	1.40bc	1.70bcd	–	72	–	–
Mech 184 + +	6.67a	4.44a	3.88a	0.00a	1.10abc	–	–	–	–	–
SE ±	4.87	5.58	4.36	5.81	0.13	0.13	–	–	–	–
LSD (p 0.05)	14.30	16.37	12.94	17.13	0.40	0.40	–	–	–	–
Fp (df)	< 0.001(11)	< 0.001(11)	< 0.001(11)	< 0.001(11)	< 0.001(11)	< 0.001(11)	–	–	–	–

[a]Duration of feeding the larvae on the respective cultivars before parasitization.

− −, *Helicoverpa armigera* larvae fed on control non-transgenic cotton leaves before and after parasitization; + −, *Helicoverpa armigera* larvae fed on leaves of transgenic cotton plants before parasitization; − +, *Helicoverpa armigera* larvae fed on leaves of transgenic cotton plants after parasitization till cocoon formation; ++, *Helicoverpa armigera* larvae fed on leaves from transgenic cotton plants before and after parasitization; *Fp*, Probability of *F*-test; df, Degrees of freedom.

Figures followed by the same letter within a column are not significantly different at $p \leq 0.05$.

larvae were fed on the leaves of non-transgenic hybrid Mech 184 (55.56–75.56%). Adults emergence was quite low (0.0–15.56%) when the parasitoid was reared on *H. armigera* larvae fed on the leaves of transgenic hybrids before and after parasitization as compared to the larvae reared on non-transgenic hybrids (42.22–93.33%). Adult emergence was also lower when the *H. armigera* larvae were fed on the leaves of transgenic plants after parasitization (4.44–22.22%). Reduction in cocoon formation and adult emergence of *C. chlorideae* reared on *H. armigera* larvae fed on the leaves of transgenic plants before and/or parasitization was because of early mortality of *H. armigera* larvae. The *H. armigera* larvae died in 6.70–8.0 days when fed on the leaves of non-transgenic hybrids, while those fed on the leaves of transgenic hybrids before and after parasitization died in 4.1–6.73 days (Table 5). However, the parasitoid egg + larval development was completed in 9.13–13.50 days on different cultivars. Therefore, early mortality of *H. armigera* was largely responsible for poor cocoon formation when the parasitoid was raised on *H. armigera* larvae fed on the leaves of transgenic plants.

Adult weight

Weight of *C. chlorideae* wasps was slightly lower when reared on *H. armigera* larvae fed on the leaves of transgenic hybrids before and after parasitization (0.98–1.70 mg) than those reared on *H. armigera* larvae fed on the leaves of non-transgenic hybrids (1.88–2.34 mg) (Table 6).

Fecundity and sex ratio

Fecundity of the females that emerged from *H. armigera* larvae fed on the leaves of transgenic hybrids was lower (66–72 cocoons per female) than that of the females emerging from *H. armigera* reared on the leaves of non-transgenic hybrid Mech 12 (123–184 cocoons per female). However, lower fecundity was also observed in case of parasitoids reared on *H. armigera* larvae fed on the leaves of non-transgenic hybrid Mech 162 (71–74 cocoons per female). In general, there were no apparent effects of transgenic plants on the sex ratio of the progeny of the parasitoids raised on transgenic plants.

Quantification of Cy1Ac toxin in cotton leaves, H. armigera *larvae, and* C. chlorideae *cocoon*

The amounts of Cry1Ac (ppm) in the leaves of transgenic hybrids varied from 0.202 to 1.287 ppm (Table 7). No *Bt* toxins were detected in the *H. armigera* larvae fed on the leaves of transgenic cotton before

Table 7. Semi-quantitative estimation of Cry1Ac toxin in leaves of transgenic cotton hybrids, *Helicoverpa armigera* larvae, and *Campoletis chlorideae* cocoons

Genotype	Cry1Ac concentration (ppm)				
	2003	2004	*Helicoverpa* larvae	*Campoletis* cocoons	Larval remnants
Mech 12	0.535	0.776	BD	BD	BD
Mech 162	0.473	0.719	BD	BD	BD
Mech 184	0.202	1.287	BD	BD	BD

BD, Below detection level.

parasitization or in cadavers after the cocoon formation. Similarly, no *Bt* toxins were detected in the cocoons of *C. chlorideae*, suggesting that reduction in cocoon formation was not due to the direct effect of *Bt* toxin on the parasitoid, but because of the early mortality of the host larvae (5–6 days) and possibly poor nutritional quality of the host.

Discussion

Transgenic cottons with *Bt* genes in combination with insecticides are quite effective for bollworm control even at lower rates of insecticide application (Brickle et al., 1999). There were no differences in the number of spiders, coccinellids, and chrysopids between transgenic and non-transgenic cultivars within a protection regime. However, there was substantial reduction in the number of generalist predators in insecticide treated plots towards the end of the cropping season. No egg and larval parasitoids were recorded in the egg and larval samples collected from the field, indicating near absence of parasitoids from the cotton eco-system at the ICRISAT farm, and this may be one of the reasons for heavy damage by *H. armigera* on cotton, pigeonpea, and chickpea in South Central India. The diversity of arthropod communities in transgenic cotton carrying *Cry1A*, *CpTi* and *Cry1Ac* genes has been found to be similar to the conventional cotton under unprotected conditions (Xin et al., 2004). The authors suggested that *Bt* cotton might increase the stability of arthropod communities in cotton eco-systems, and result in sustainable pest management in this crop. No major differences have been observed in the abundance of predators in fields with transgenic and non-transgenic crops (Hoffmann et al., 1992; Sims, 1995; Flint et al., 1995;

Luttrell et al., 1995; Orr and Landis, 1997; Daly and Buntin, 2005; Head et al., 2005; Torres and Ruberson, 2005). However, *Bt* toxin Cry1Ab has been detected in predatory arthropods in corn (Harwood et al., 2005) and in the ground beetles collected from fields with *Bt* corn residues (Zwahlen and Andow, 2005). There is therefore, a need to study the implications of exposure of non-target organisms to *Bt* toxins from the transgenic crops. The *Bt* toxins have also been detected in the non-target herbivores such as *Chaetocnema pulicaria* (Melsh.), *Popillia japonica* (Newman), and *Diabrotica undecimpunctata howardi* Barber (Harwood et al., 2005). Adverse effects of microbially produced *Bt* toxins have been observed on *C. carnea* when mixed into artificial diet (Hilbeck et al., 1999). Some reduction in the fitness of the predatory chrysopid larvae was also attributable to caterpillars fed on *Bt*-maize pollen (Hoffmann et al., 1992; Hilbeck et al., 1998a, b, 1999). However, Romeis et al. (2004) reported that *C. carnea* larvae ingesting 10,000 times more Cry1Ab showed no adverse effects on its survival and development. Significant increase in mortality of *C. carnea* larvae has been observed when fed on *Bt* contaminated *Spodoptera littoralis* Boisd. (Dutton et al., 2002, 2003). Hagerty et al. (2005) reported that populations of the predators (*Geocoris* sp., *Orius insidiosis* (Seg.), *Nabis* spp., *Solenopsis invicta* Buron, spiders, coccinellids, and lacewings were at par or high in Bollgard I and Bollgard II transgenic cottons as compared to the non-transgenic ones.

There was a slight (2–3 days) prolongation of the development period of *C. chlorideae* when raised on *H. armigera* larvae fed on the leaves of transgenic cotton before and/or after parasitization. Egg and larval periods of *C. chlorideae* were also prolonged when the host larvae were fed on the leaves of cotton hybrid, Mech 184. There was a significant reduction in cocoon formation and adult emergence when the parasitoid was raised on *H. armigera* larvae fed on leaves of the transgenic plants before and after parasitization. Reduction in cocoon formation and adult emergence of *C. chlorideae* reared on *H. armigera* larvae fed on the leaves of transgenic plants before and/or parasitization was not because of adverse effects of the *Bt* toxins on the parasitoid but due to early mortality of *H. armigera* larvae caused by *Bt* toxins. Under natural conditions, the *H. armigera* larvae feed on cotton leaves occasionally, and therefore, there is a need to compare the parasitoid survival and development on *H. armigera* larvae raised on squares and bolls, the preferred plant parts. There were no trends in sex ratio of the *C. chlorideae* wasps emerging from *H. armigera* larvae fed on the leaves of transgenic or non-transgenic hybrids, while a

slight reduction in fecundity of the females was recorded on some transgenic hybrids.

The present studies showed that there were considerable adverse effects of the transgenic cotton on the parasitoid, *C. chlorideae* through early mortality of host larvae, and sub-optimal prey because of slow growth of the larvae. Lu et al. (2004) also observed a reduction in cocoon formation and weight of *M. mediator* and *C. chlorideae* reared on *H. armigera* fed on transgenic cotton. Under field conditions, parasitization of European corn borer, *Ostrinia nubilalis* (Hubner) by the host specific parasitoid, *Macrocentrus cingulum* Brischke is reduced significantly (Pilcher et al., 2005). Some of the predatory arthropods might be exposed to the *Bt* toxins from the herbivores, and such an information should be taken into consideration in long-term studies on non-target effects of transgenic crops (Harwood et al., 2005). Long-term assessment of the effect of *Bt* cotton on non-target arthropod natural enemies indicated 19% reduction in major arthropods in unsprayed fields, which was linked to reduction in prey in the *Bt* cotton fields (Naranjo, 2005). Studies under field conditions have shown that transgenic plants and the parasitoid wasp, *C. sonorensis* act synergistically, decreasing the survival of *H. virescens* larvae beyond the level expected for an additive interaction. Synergistic increases in mortality and parasitism of the host larvae have been observed when development rates on toxic plants and control plants were equal (Johnson and Gould, 1992). Egg parasitism of third-generation noctuids on *Bt*-transgenic cotton has been found to be lower than in the conventional cottons (Wang and Xia, 1997). In natural and integrated control plots, the numbers of *C. chlorideae* and *Microplitis* sp. decreased by 79.2 and 87.5, and 88.9 and 90.7%, respectively (Cui and Xia, 1997, 1998).

The effects of transgenic crops on the natural enemies vary across crops and the cropping systems. Some of the variation may be due to differences in pest abundance between the transgenic and the non-transgenic crops. In general, there were no adverse effects of transgenic cotton on the relative abundance of coccinellids, spiders, and chrysopids under field conditions. There was a significant reduction in survival of the host specific parasitoid, *C. chlorideae* raised on *H. armigera* larvae fed on the leaves of transgenic cotton before and after parasitization largely because of early mortality of the *H. armigera* larvae due to *Bt* toxins. Prolonged feeding of *H. armigera* larvae on cotton leaves also produced adverse effects on survival and development of *C. chlorideae*, and that such effects were greater for

Mech 184 than for Mech 12. Therefore, it would be useful to compare the survival and development of the *C. chlorideae* raised on *H. armigera* larvae fed on the leaves, flowers, and bolls of different cotton genotypes to develop appropriate strategies for deployment of transgenic crops for integrated pest management and sustainable crop production.

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