JOURNAL OF APPLIED ENTOMOLOGY
J. Appl. Entomol.

Chickpea-mediated effects of *Bacillus thuringiensis* on *Helicoverpa armigera* and its larval parasitoid, *Campoletis chlorideae*

M. K. Dhillon & H. C. Sharma

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India

Keywords
Bacillus thuringiensis, *Campoletis chlorideae*, *Helicoverpa armigera*, chickpea, non-target effects, tritrophic interactions

Correspondence
Hari C. Sharma (corresponding author), Principal Scientist – Entomology, ICRISAT, Patancheru 502 324, Andhra Pradesh, India.
E-mail: h.sharma@cgiar.org

Received: August 5, 2009;
accepted: January 7, 2010.

doi: 10.1111/j.1439-0418.2010.01506.x

Abstract

Efforts are underway to express toxin genes from *Bacillus thuringiensis* (*Bt*) in chickpea for controlling the pod borer, *Helicoverpa armigera*. The interaction between *Bt* toxins, *Helicoverpa*-resistant chickpeas, and the parasitoid, *Campoletis chlorideae* are not fully understood. Therefore, we studied tritrophic interactions between *Bt* (administered as spray), chickpea genotypes, and the parasitoid, *C. chlorideae*. Chickpea genotypes resistant to *H. armigera* exercised a significant reduction in leaf feeding, survival and development of *H. armigera*, but did not influence the development and survival of the parasitoid, *C. chlorideae*. *Bt* sprays on different chickpea genotypes prolonged the larval period, and reduced pupation and adult emergence of *C. chlorideae*. Weights of *H. armigera* larvae showed a strong and positive association with *C. chlorideae* larval period on *Bt* treated, and a negative association on untreated chickpeas. The *Bt*-intoxicated *H. armigera* larvae also resulted in reduced weight of the cocoons and adults of *C. chlorideae*, suggesting significant influence of host size on development and survival of the parasitoid. *Bt* toxins were detected in *H. armigera* larvae fed on *Bt*-sprayed chickpeas, but not in *C. chlorideae* reared on *H. armigera* larvae fed on *Bt*-treated chickpeas, and in the parasitoid adults fed on honey intoxicated with 0.05% *Bt*. The adverse effects of *Bt* on the parasitoid were largely through early mortality of *H. armigera* larvae or poor quality of the host. This information would be useful for planning appropriate strategies for testing and deployment of *Bt*-transgenic chickpea with resistance to *H. armigera* for sustainable crop production.

Introduction

Chickpea (*Cicer arietinum* L.) is the world's third most important food legume, grown in tropical, subtropical and temperate regions, and is the premier pulse crop in the Indian subcontinent (Hulse 1991). It is valued for its nutritive seeds with high protein content (25.3–28.9%). Chickpea seeds are consumed fresh as a green vegetable, fried, roasted and boiled as a snack food. The grain after dehulling is largely consumed as split seeds as 'dhal', or the split seeds are ground as flour, which is used to make bread, snacks and sweets. The straw is used as feed for livestock. It is grown on about 10.38 million ha with a production of 8.57 million tonnes worldwide (FAO 2004). India is the largest producer as well as consumer of chickpea. In India, chickpea is grown on about 6.67 million ha with a production of 5.3 million tonnes (Majumder 2009). Chickpea yields are low (400–600 kg/ha), because of several biotic and abiotic constraints, of which the pod borer, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera) is

the most important constraint in chickpea production (Manjunath et al. 1989). In addition to chickpea, *H. armigera* also damages several other crops such as cereals, pulses, cotton, vegetables, fruit crops and forest trees. It causes an estimated loss of US$2 billion annually, despite US$500 million worth of insecticides used to control this pest worldwide (Sharma 2005).

The parasitic wasp, *Campoletis chlorideae* Uchida (Ichneumonidae: Hymenoptera), parasitizes several lepidopteran insect species (Yan and Wang 2006; Dhillon and Sharma 2007), and is one of the common larval parasitoids of the pod borer, *H. armigera* in chickpea (Bhatnagar et al. 1982; Kumar et al. 1994). *Bacillus thuringiensis* (Berliner) (*Bt*) has been used extensively for the management of *H. armigera* in India, China, Philippines, Malaysia and North America (Gujar 2005). It can be used in combination with conventional host-plant resistance for managing this pest. In addition to the use of *Bt* as a conventional pesticide, *Bt*-transgenic crops, which constitutively produce δ-endotoxins from *Bt*, can be used to provide protection from insect damage throughout the crop season. Transgenic cottons with *Bt* toxin genes have been released for cultivation in several countries (James 2007), whereas transgenic chickpea with *Bt* genes expressing either Cry1Ac or Cry2Aa, or both proteins, are currently under development and could become commercially available for imparting resistance to *H. armigera* (Sanyal et al. 2005; Sharma et al. 2005a,b; McPhee et al. 2007). Considerable information is available on the host-mediated effects of *Bt*-transgenic crops on the parasitoids (Romeis et al. 2006). Although, *Bt*-transgenic chickpea has been found compatible with entomopathogenic fungus, *Metarhizium anisopliae* for the management of *H. armigera* (Lawo et al. 2008), compatibility of *Bt*-chickpea with *H. armigera* larval parasitoid, *C. chlorideae* has not been tested yet, which might influence its activity and abundance in the chickpea ecosystem. The effects of *Bt* toxins on the parasitoid could be due to direct exposure to the toxins through *Bt* spray or *Bt*-contaminated chickpea leaf exudates or honeydew from aphids, and the indirect effects via reduction in host density and nutritional quality. Moreover, interaction between *Bt* toxins and chickpea genotypes with different levels of resistance to *H. armigera*, and the parasitoid, *C. chlorideae*; and the adverse effects of direct exposure of adult parasitoids to *Bt* toxins on longevity, fitness and fecundity are also not fully understood. Therefore, the present studies were undertaken to investigate the direct effects of *Bt* on *C. chlorideae* through *Bt*-contaminated honey, and indirect effects through chickpea genotypes with different levels of resistance to *H. armigera* and *Bt* sprays on the survival, development and fecundity of the parasitoid, *C. chlorideae*.

Materials and Methods

Plant material

Four chickpea genotypes (ICC 506 – resistant, ICCV 10 – moderately resistant, C 235 – moderately susceptible and L 550 – susceptible) were planted during the 2005–2006 and 2007–2008 post-rainy seasons (October–March) at the research farm of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Recommended agronomic practices, except insecticide sprays, were followed for raising the crop. Each genotype was planted in a four-row plot, 2 m long, and the rows were 60 cm apart. There were three replications in a randomized complete block design. The experiment was planted in two sets, on an area of 16 m^2 each. The test plots were covered with a nylon net to avoid interference from other insect species, and natural infestation by *H. armigera*. One set of chickpea genotypes was sprayed with a sublethal dose (0.05%) of *Bt* (Biolep®; Biotech International Ltd., New Delhi, India) at the flowering stage with knapsack sprayer, while the unsprayed genotypes were used as controls.

Characteristics of *Bt* formulation (Biolep®)

The *Bacillus thuringiensis* var. *kurstaki* (Serotype H-3 a, 3 b, Strain Z-52) formulation Biolep® used in the present studies, is a water dispersible powder, which acts on the host larvae through its parasporal crystal δ-endotoxins and the bacterial spores. Biolep® contained 5–8% *Bt* δ-endotoxins, 5–8% *Bt* spores, 37–55% nutrient medium residues, 15–20% sodium chloride, 15–18%, fillers (Kaolin) and a moisture content of 5–9%. The *Bt* formulation sprayed on the chickpea plants or mixed in 10% honey solution [0.05%, i.e. ED_{75} (effective concentration to cause a 75% reduction in *H. armigera* larval weight)], contained 25–40 μg/ml *Bt* δ-endotoxins, and 25–40 μg/ml *Bt* spores. It is a mixture of Cry1Aa (28%), Cry1Ab (53%), Cry1Ac (19%), and Cry2A and Cry2B (<0.1%) (Chandrashekar et al. 2005).

Insect culture

Helicoverpa armigera larvae were reared on chickpea-based semi-synthetic artificial diet under laboratory conditions at 27 ± 2°C and 65–85% RH (Armes et al. 1992). The *H. armigera* culture maintained in the laboratory was used for rearing the parasitoid, *C. chlorideae*, and for conducting the bioassays.

Cocoons of *C. chlorideae* were collected from chickpea fields at the ICRISAT research farm, Patancheru, India, and placed individually in plastic vials (2.5 cm diameter × 7.5 cm height) until adult emergence. The adult wasps were released in plastic cages (15 cm diameter × 18.5 cm height) for mating, and fed *ad libitum* on 10% honey solution. The mating was observed visually, and the mated pairs were transferred to another cages. For oviposition, the mated females were transferred to transparent plastic vials (2.5 cm diameter × 7.5 cm height) kept in an inverted condition in a Petri dish (9.5 cm diameter × 1 cm height). Single second-instar larvae of *H. armigera* were offered to the parasitoid females for female attack. The parasitoid females, in general, attacked the *H. armigera* larvae in 1–2 min. After female attack, the *H. armigera* larvae were removed, and placed on chickpea-based artificial diet for further development. The parasitoid culture and the bioassays were conducted at 27 ± 2°C, 65–75% RH and a 12-h photoperiod in the laboratory.

Effects of *Bacillus thuringiensis*-treated chickpeas on *Helicoverpa armigera* larvae

Host-plant-mediated effects of *Bt* on leaf feeding, survival and development of *H. armigera* larvae were studied on four chickpea genotypes. Terminal branches from the *Bt*-sprayed (after 2 h of spraying) and unsprayed plants were brought to the laboratory, and 10 neonate *H. armigera* larvae were released on each branch (having four leaves and a growing tip) using detached leaf bioassay (Sharma et al. 2005a,b). There were 8 and 10 replications (number of terminal branches bioassayed for each genotype; N = 18) during the 2005–2006 and 2007–2008 post-rainy seasons (October–March), respectively, in a completely randomized design (CRD). After 5 days of feeding, the *Bt*-sprayed and unsprayed chickpea branches were evaluated for leaf damage on a 1–9 scale (1 ≤ 10% leaf area damaged, and 9 ≥ 80% leaf area damaged). The surviving larvae were individually collected in 25-ml cups and weighed (using Mettler AE 160 balance; Mettler-Toledo Inc., Columbus, OH, USA) after 4 h to assess weight gain by the larvae.

Host-plant-mediated effects of *Bt* sprays on the larval parasitoid, *Campoletis chlorideae*

Host-plant-mediated effects of *Bt* sprays on survival and development of *C. chlorideae* were studied through *H. armigera* larvae fed on four *Bt*-sprayed/unsprayed chickpea genotypes during the 2005–2006 and 2007–2008 post-rainy seasons. Neonate *H. armigera* larvae were fed on terminal branches of *Bt*-sprayed and control chickpeas using the detached leaf bioassay. After 5 days of feeding on the *Bt*-sprayed foliage, the *H. armigera* larvae were exposed to the *C. chlorideae* females till they attacked the host larvae, and were again fed on the *Bt*-sprayed foliage of the same chickpea genotype till emergence of the parasitoid larvae from the host larvae. In the case of larvae fed on unsprayed foliage, the larvae were exposed for parasitization after 3 days, as they grew at a faster rate. Twenty-five *H. armigera* larvae were parasitized per replication, and there were three replications in a CRD in each season (N = 6). Observations were recorded on percent parasitization, days to cocoon formation (egg + larval period), pupal period, cocoon formation and adult emergence. The weight, length and diameter of the parasitoid cocoons and weights of adult males and females were also recorded. The cocoons and adults were weighed using Mettler AE 160 balance, and the length and diameter of the cocoons was recorded using vernier calipers. Live parasitoid adults were weighed within 24 h of emergence. The parasitoid adults were collected individually in the plastic vials with the help of a specially designed aspirator, to avoid escape during the weighing. Fecundity (equivalent to number of stabbings/female; Dhillon and Sharma 2009b) of *C. chlorideae* females emerging from the larvae fed on *Bt*-treated and untreated chickpea genotypes was recorded for five pairs (N = 5), and the carry-over effects of *Bt* and/or chickpea genotypes on the parasitoid survival and development were studied by rearing their progenies on the *H. armigera* larvae fed on control artificial diet at 27 ± 2°C, 65–75% RH, and a 12-h photoperiod in the laboratory.

Direct effects of *Bt* on *Campoletis chlorideae* adults

The direct effects of *Bt* (*Bt* was mixed at 0.05% in 10% honey solution) were assessed on the male and female longevity and fecundity, and on cocoon formation and adult emergence of the progenies through *Bt*-contaminated 10% honey solution fed to the adults of *C. chlorideae*. The honey solution was

changed on alternate days. The adults fed on uncontaminated 10% honey were used as controls. Six adult males and females were used per treatment in this experiment, thus making six replications (N = 6) in a CRD. The experiment was conducted under laboratory conditions at 27 ± 2°C, 65–75% RH, and a 12-h photoperiod.

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

Helicoverpa armigera larvae fed on *Bt*-sprayed and unsprayed chickpea plants, and larvae, pupae, and adults of *C. chlorideae* reared on *H. armigera* larvae fed on *Bt*-sprayed and unsprayed plants, as well as the *C. chlorideae* adults fed on *Bt*-contaminated honey were subjected to a semi-quantitative ELISA test (Agdia®, Inc., Elkhart, IN, USA) for detection of Cry1Ab and Cry1Ac *Bt* toxins. However, other *Bt* toxins present in the *Bt* formulation Biolep® could not be detected/assessed through ELISA kit as we were not having access to antibodies for Cry1Aa, Cry2A and Cry2B. These toxins though present in very small amounts (except Cry 1Aa), may be taken into consideration while doing such bioassays. *Helicoverpa armigera* larvae fed on *Bt*-sprayed chickpeas and the *C. chlorideae* adults fed on *Bt*-contaminated honey were washed thoroughly with PBS buffer to avoid *Bt* contamination of the insect samples through contact with their food. *Campoletis chlorideae* larvae were collected from parasitized *H. armigera* larvae when the parasitoid larvae were ready to emerge from the host larvae for pupation. In each replication, 8–10 *H. armigera* larvae/different life stages of the parasitoid were collected in separate Eppendorf tubes and crushed in PBS buffer in the ratio of 1 : 10 (insect sample: buffer). The ELISA test was performed for each sample as reported by Sharma et al. (2008).

Statistical analysis

The data were subjected to normality and homogeneity tests, and the seasonal effects were found to be non-significant. Thus, data from both the seasons were pooled for analysis of variance (ANOVA) using GenStat 10th version (GenStat 2008) in a factorial design to test the effects of genotypes, *Bt* sprays, and the interaction effects of genotypes × *Bt* sprays on different life stage parameters of *C. chlorideae*. The significance of differences between the treatments and their interaction effects were judged by *F*-test, while the treatment mean values were compared by least significant difference (LSD) at $P \leq 0.05$. Association between size of the host, *H. armigera* larvae and parasitoid performance on different chickpea genotypes under *Bt*-sprayed and unsprayed conditions were analysed using scatter plots showing regression lines.

Results

Effect of chickpea genotypes and *Bt* sprays on leaf feeding, and survival and development of *Helicoverpa armigera*

Survival of *H. armigera* larvae was significantly influenced by both *Bt* spray ($F_{1,17} = 165.41$, $P < 0.001$) and the chickpea genotypes ($F_{3,51} = 2.78$, $P = 0.04$), but the interaction effects were non-significant. However, the interaction effects of *Bt* sprays × chickpea genotypes for leaf damage ($F_{3,51} = 8.48$, $P < 0.001$) and larval weight ($F_{3,51} = 6.70$, $P < 0.001$) were significant. Foliar damage by *H. armigera*, and the larval survival and weights were significantly lower on *Bt*-sprayed plants than on the unsprayed plants (table 1). Leaf damage, and larval survival and weights were significantly greater on L 550 (susceptible) than that on ICC 506 (resistant) under untreated conditions. However, these genotypic effects were not apparent when the plants were sprayed with *Bt*.

Host-mediated effects of *Bt* sprays on *Campoletis chlorideae*

The parasitoid, *C. chlorideae* larval period was significantly prolonged ($F_{1,5} = 72.38$, $P < 0.001$) in insects reared on *H. armigera* larvae fed on *Bt*-treated chickpeas as compared to the untreated controls (table 2). The larval period of *C. chlorideae* in *Bt*-treated chickpeas fed *H. armigera* larvae was prolonged by <1 day. Cocoon formation ($F_{1,5} = 403.86$, $P < 0.001$) and adult emergence ($F_{1,5} = 421.54$, $P < 0.001$) were significantly lower in *C. chlorideae* reared on *H. armigera* larvae fed on *Bt*-treated chickpeas as compared to those fed on untreated controls (table 2). There were no significant effects of *Bt* sprays or genotypes on the pupal period of *C. chlorideae*. *Helicoverpa armigera* larvae fed on *Bt*-sprayed chickpeas reduced cocoon formation (by 42.6–56.0%), and adult emergence (by 40.0–52.0%) of *C. chlorideae* over the untreated controls (table 2). There were no significant effects of chickpea genotypes on the development period, cocoon formation and adult emergence of *C. chlorideae*.

Table 1 Host-plant-mediated effects of *Bacillus thuringiensis* on leaf feeding, and survival and development of *Helicoverpa armigera*

	Damage rating[1]			Larval survival (%)			Larval weight (mg/larva)		
Chickpea genotypes	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean
L 550	1.9 a	6.6 b	4.2	27.2 a	78.9 b	53.1	1.25 a	5.10 b	3.17
C 235	2.5 a	5.2 b	3.9	35.6 a	71.1 b	53.3	1.31 a	5.61 b	3.46
ICCV 10	2.3 a	5.2 b	3.7	26.7 a	66.1 b	46.4	1.43 a	5.54 b	3.48
ICC 506	1.7 a	3.4 b	2.5	26.1 a	59.4 b	42.8	1.34 a	3.65 b	2.49
Mean	2.1	5.1	–	28.9	68.9	–	1.3	5.0	–
For comparing	SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)	
Genotype (G)	0.21	0.60**		3.11	8.71*		0.18	0.49**	
Bt spray (T)	0.15	0.43**		2.20	6.16**		0.12	0.35**	
G × T	0.30	0.85**		4.40	NS		0.25	0.70**	

*, **, Significant at P ≤ 0.05 and 0.01, respectively. NS, non-significant at P ≤ 0.05.
[1]Damage rating (1 ≤ 10% leaves were damaged, and 9 ≥ 80% leaves were damaged). The values for *Bt* sprayed and untreated controls for a genotype under each parameter following different letters are significant at P ≤ 0.05.

Table 2 Effect of *Bacillus thuringiensis*-treated chickpea fed to *Helicoverpa armigera* larvae on the survival and development of *Campoletis chlorideae* in the first generation

	Larval period (days)			Pupal period (days)			Cocoon formation (%)			Adult emergence (%)		
Chickpea genotypes	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean
L 550	7.6 b	7.1 a	7.3	5.8 a	5.6 a	5.7	26.7 a	69.3 b	48.0	16.0 a	56.0 b	36.0
C 235	7.7 b	7.0 a	7.4	5.7 a	5.7 a	5.7	22.0 a	74.7 b	48.3	15.3 a	55.3 b	35.3
ICCV 10	8.0 b	7.1 a	7.6	5.5 a	5.6 a	5.6	22.7 a	78.7 b	50.7	14.0 a	60.0 b	37.0
ICC 506	7.8 b	7.2 a	7.5	5.9 a	5.7 a	5.8	28.0 a	75.3 b	51.7	14.0 a	66.0 b	40.0
Mean	7.8	7.1	–	5.7	5.7	–	24.8	74.5	–	14.8	59.3	–
For comparing	SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)	
Genotype (G)	0.08	NS		0.06	NS		2.47	NS		2.17	NS	
Bt spray (T)	0.06	0.17**		0.04	NS		1.75	5.02**		1.53	4.40**	
G × T	0.12	NS		0.09	NS		3.50	NS		3.07	NS	

*, **, Significant at P ≤ 0.05 and 0.01, respectively. NS, non-significant at P ≤ 0.05. The values for *Bt* sprayed and untreated controls for a genotype under each parameter following different letters are significant at P ≤ 0.05.

Host-plant-mediated effects of *Bt* on morphological traits of *Campoletis chlorideae*

The effects of chickpea genotypes and *Bt* sprays were significant on cocoon weight (genotypes: $F_{3,15} = 5.26$, P = 0.004; *Bt* sprays: $F_{3,15} = 223.5$, P < 0.001), length (genotypes: $F_{3,15} = 9.25$, P < 0.001; *Bt* sprays: $F_{3,15} = 176.66$, P < 0.001) and diameter (genotypes: $F_{3,15} = 4.21$, P = 0.012; *Bt* sprays: $F_{3,15} = 63.91$, P < 0.001) of the parasitoid, *C. chlorideae*. However, the interaction effects of chickpea genotypes × *Bt* sprays on these cocoon characteristics were non-significant at P = 0.05. Cocoon weight, length, and diameter of the parasitoid were significantly reduced when reared on *H. armigera* larvae fed on the resistant genotype, ICC 506 (table 3). The cocoons weighed lower (7.11 vs. 10.06 mg/cocoon), and were smaller in length (4.98 vs. 5.89 mm) and breadth (2.59 vs. 2.97 mm) when reared on *Bt*-sprayed chickpea-fed *H. armigera* larvae as compared to the ones reared on untreated chickpeas (table 3).

The interaction effects of *Bt* sprays × chickpea genotypes on male parasitoid body weight ($F_{3,12} = 6.78$, P = 0.001) and longevity ($F_{3,12} = 3.03$, P = 0.048) were significant. The *Bt* sprays also reduced the weight of *C. chlorideae* females significantly ($F_{1,4} = 40.38$, P < 0.001). The males and females obtained from *H. armigera* larvae fed on

Table 3 Effects of *Bacillus thuringiensis*-treated chickpea fed to *Helicoverpa armigera* larvae on the size and weight of *Campoletis chlorideae* cocoons

	Weight (mg)			Length (mm)			Diameter (mm)		
Chickpea genotypes	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean
L 550	7.73 a	9.83 b	8.78	5.07 a	6.00 b	5.53	2.57 a	3.00 b	2.78
C 235	7.01 a	10.63 b	8.82	5.21 a	6.03 b	5.62	2.79 a	3.01 b	2.90
ICCV 10	7.24 a	10.41 b	8.83	4.91 a	6.01 b	5.46	2.53 a	3.01 b	2.77
ICC 506	6.46 a	9.36 b	7.91	4.75 a	5.54 b	5.14	2.46 a	2.86 b	2.66
Mean	7.11	10.06	–	4.98	5.89	–	2.59	2.97	–
For comparing	SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)	
Genotype (G)	0.20	0.57*		0.07	0.20**		0.05	0.14*	
Bt spray (T)	0.14	0.40**		0.05	0.14**		0.03	0.10**	
G × T	0.28	NS		0.10	NS		0.07	NS	

*, **, Significant at P ≤ 0.05 and 0.01, respectively. NS, non-significant at P ≤ 0.05. The values for *Bt* sprayed and untreated controls for a genotype under each parameter following different letters are significant at P ≤ 0.05.

Table 4 Effects of *Helicoverpa armigera* larvae fed on *Bacillus thuringiensis*-treated chickpea on the longevity and weight of *Campoletis chlorideae* adults

	Longevity (days)			Weight (mg)		
Chickpea genotypes	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean
Male						
L 550	10.8 a	12.0 a	11.4	2.45 a	2.78 b	2.61
C 235	16.8 a	13.3 a	15.1	2.18 a	3.03 b	2.60
ICCV 10	12.6 a	12.8 a	12.7	1.38 a	2.95 b	2.16
ICC 506	11.2 a	14.3 a	12.8	1.68 a	2.49 b	2.09
Mean	12.9	13.1	–	1.92	2.81	–
For comparing	SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)	
Genotype (G)	0.80	2.33*		0.10	0.28**	
Bt spray (T)	0.57	NS		0.07	0.20**	
G × T	1.13	3.29*		0.14	0.40*	
Female						
L 550	17.0 a	16.0 a	16.5	2.54 a	3.53b	3.04
C 235	15.7 a	18.8 a	17.3	2.27 a	3.29b	2.78
ICCV 10	17.0 a	15.9 a	16.5	2.06 a	3.53 b	2.80
ICC 506	17.2 a	18.9 a	18.1	2.66 a	3.20 b	2.93
Mean	16.7	17.4	–	2.38	3.39	–
For comparing	SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)	
Genotype (G)	0.85	NS		0.16	NS	
Bt spray (T)	0.60	NS		0.11	0.32**	
G × T	1.21	NS		0.22	NS	

*, **, Significant at P ≤ 0.05 and 0.01, respectively. NS, non-significant at P ≤ 0.05. The values for *Bt* sprayed and untreated controls for a genotype under each parameter following different letters are significant at P ≤ 0.05.

unsprayed chickpeas were heavier (2.81 mg/male and 3.39 mg/female) than those obtained from *Bt*-sprayed chickpeas (1.92 mg/male and 2.38 mg/female) (table 4). However, the effects of *Bt* sprays on the longevity of parasitoid males and females were not significant.

The scatter plots showed a significant and positive association between *H. armigera* larval weights

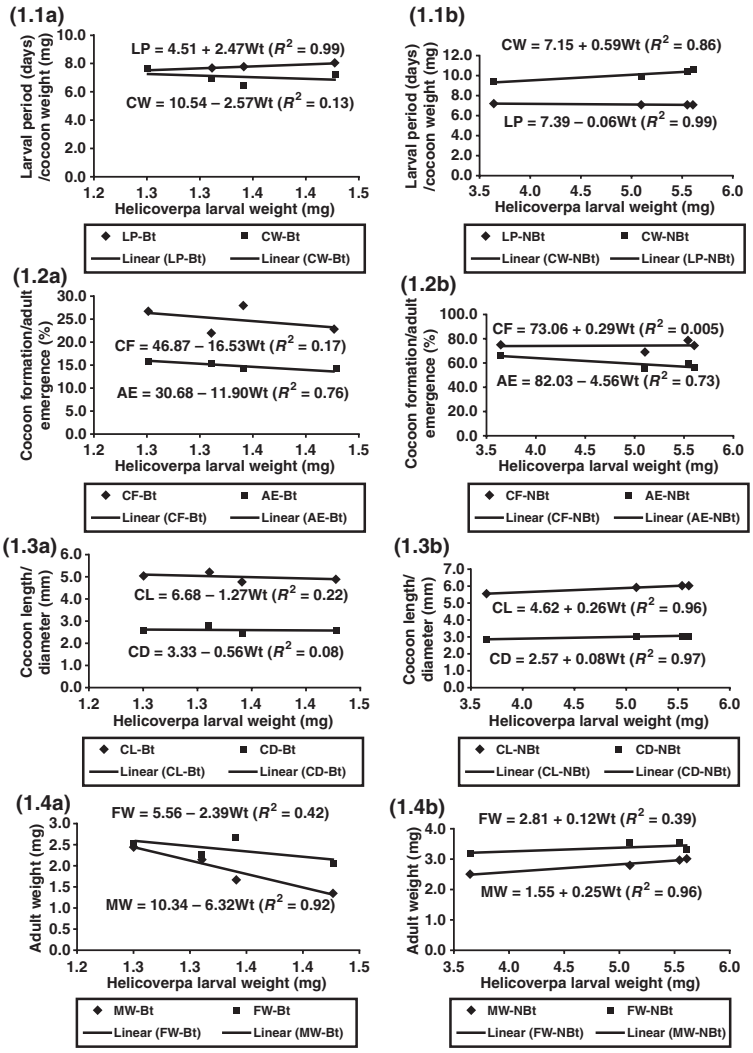

Fig. 1 Scatter plots showing regression equations of *Campoletis chlorideae* larval period (LP) and cocoon weight (CW) (1.1a, 1.1b), cocoon formation (CF) and adult emergence (AE) (1.2a, 1.2b), cocoon length (CL) and diameter (CD) (1.3a, 1.3b), and adult male (MW) and female (FW) weights (1.4a, 1.4b) with respect to *Helicoverpa armigera* larval weights (WT) in *Bacillus thuringiensis*-sprayed (BT) and unsprayed (NBT) chickpea genotypes, respectively.

and *C. chlorideae* larval period on *Bt*-treated chickpeas (fig. 1.1a), whereas a negative association was observed on untreated chickpeas (fig. 1.1b). However, reverse was the trend for cocoon weights with significant association under unsprayed and weak association under *Bt*-sprayed conditions, as the range in host larval weights was very narrow (1.3–1.5 mg) on *Bt*-sprayed chickpeas (figs. 1.1a,b). Cocoon formation was not influenced by the size of the *H. armigera* larvae on different chickpea genotypes with and without *Bt* treatment, but adult emergence was significantly and negatively influenced by the weight of insect host larvae (fig. 1.2a,b). The cocoon length and diameter were not significantly influenced by the size of *H. armigera* larvae on chickpeas treated with *Bt* (fig. 1.3a), but a positive effect of the host, *H. armigera* larvae was observed on cocoon size under unsprayed conditions (fig. 1.3b). The parasitoid female adult weights were poorly associated with the weight of the *H. armigera* larvae, but a significant and negative association was observed between male adult weights and the weights of *H. armigera* larvae under *Bt* treated (fig. 1.4a), whereas positively associated under untreated conditions (fig. 1.4b). This may be because of smaller size of the males, which may render them more sensitive to changes in host larvae.

Carry-over effects of *Bt* sprays on the *Campoletis chlorideae* in the next generation

The interaction between chickpea genotypes × *Bt* spray showed a significant influence on cocoon formation ($F_{3,12}$ = 7.26, P = 0.001) and adult emergence ($F_{3,12}$ = 9.85, P < 0.001) in the following

Table 5 Influence of *Bacillus thuringiensis*-treated chickpea fed to *Helicoverpa armigera* larvae on the survival and development of *Campoletis chlorideae* in the second generation

	Cocoon formation (%)			Adult emergence (%)			Fecundity/female			Sex ratio (M : F)		
Chickpea genotypes	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean	*Bt* sprayed	Untreated control	Mean
L 550	55.6 a	63.0 b	59.3	45.1 a	51.0 b	48.0	140.6 b	108.5 a	124.6	0.89 a	0.68 a	0.78
C 235	82.6 b	72.7 a	77.7	69.6 b	58.9 a	64.2	126.6 a	128.6 a	127.6	0.64 a	0.90 a	0.77
ICCV 10	78.0 b	64.9 a	71.5	68.7 b	49.3 a	59.0	141.0 b	109.3 a	125.2	0.67 a	0.60 a	0.63
ICC 506	66.4 a	64.9 a	65.7	54.7 a	54.8 a	54.8	143.0 a	126.4 a	134.7	0.71 a	0.85 a	0.78
Mean	70.7	66.4	–	59.5	53.5	–	137.8	118.2	–	0.73	0.75	–
For comparing	SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)		SE±	LSD (P ≤ 0.05)	
Genotype (G)	1.70	4.96**		1.79	5.21**		9.46	NS		0.14	NS	
Bt spray (T)	1.20	3.51*		1.27	3.68*		6.69	19.49*		0.10	NS	
G × T	2.41	7.01**		2.53	7.37**		13.38	NS		0.20	NS	

*, **, Significant at P ≤ 0.05 and 0.01, respectively. NS, non-significant at P ≤ 0.05. The values for *Bt* sprayed and untreated controls for a genotype under each parameter following different letters are significant at P ≤ 0.05.

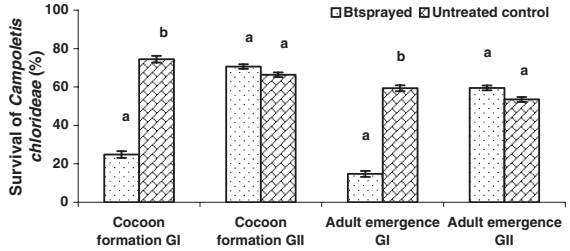

Fig. 2 Effect of *Bacillus thuringiensis* sprays on chickpea, via the host *Helicoverpa armigera*, on survival and progeny production of *Campoletis chlorideae* for two generations (GI, Generation I; GII, Generation II).

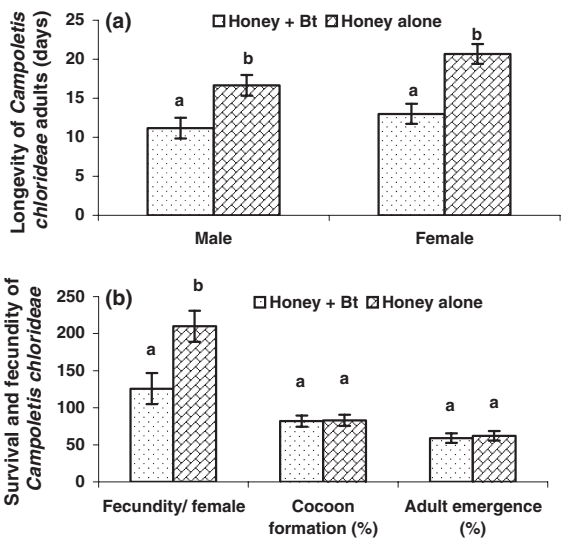

Fig. 3 Effects of *Bacillus thuringiensis* in honey solution fed to adults of *Campoletis chlorideae* on their longevity (a), and survival and fecundity (b).

generation (table 5; fig. 2). Cocoon formation and adult emergence in *C. chlorideae* parasitizing *H. armigera* fed on *Bt*-treated ICCV 10 and C 235 was greater than that on ICC 506 and L 550 (table 5). Fecundity of *C. chlorideae* females obtained from *Bt*-treated chickpea-fed *H. armigera* (137.8 stabbings/female) was significantly greater than those reared on the untreated chickpeas (118.2 stabbings/female) ($F_{1,4}$ = 4.29, P = 0.049). There were no significant effects of chickpea genotypes on the fecundity of *C. chlorideae* females in the second generation (table 5).

Direct effects of *Bt* on *Campoletis chlorideae* adults

Direct exposure of the parasitoid adults to *Bt* in 10% honey significantly reduced the male ($F_{1,5}$ = 8.60, P = 0.033) and female ($F_{1,5}$ = 18.89, P = 0.007) longevity, and fecundity ($F_{1,5}$ = 8.04, P = 0.036). The *Bt*-treated honey reduced male and female longevity by 5.5 and 7.7 days, respectively (fig. 3a), and fecundity by 40.0% (fig. 3b). However, there were no significant effects of *Bt* on cocoon formation and adult emergence of the progeny (fig. 3b).

Presence of *Bt* toxins in insect host and the parasitoid

The ELISA test detected >5 ppb of *Bt* toxins in *Bt*-sprayed chickpea genotypes, and the *H. armigera* larvae fed on them. However, no *Bt* toxins were detected in the larvae, cocoons and adults of

C. chlorideae reared on *Bt*-intoxicated *H. armigera* larvae, or in adult parasitoids fed on *Bt*-contaminated honey.

Discussion

Insect–host-plant interactions are critical in determining the effectiveness of natural enemies for biological control of insect pests. Synergism between host-plant resistance and biological control is an important phenomenon for developing practical and effective strategies for pest management. Spraying *Bt* onto resistant as well as susceptible chickpea genotypes significantly reduced the survival of, and damage by *H. armigera* larvae as compared to that on the unsprayed controls, suggesting that resistant genotypes are compatible with *Bt* sprays for the management of *H. armigera*. In certain cases, the secondary metabolites that impart resistance to insects are compatible with the natural enemies (Starks et al. 1972; Starks and Burton 1977; Barbosa et al. 1986). *Campoletis sonorensis* (Cameron) females attack early instars of *Helicoverpa virescens* (Fab.), but do not attack the bigger larvae. Therefore, under a low level of antibiosis in moderately resistant plants, the larvae of *H. virescens* remain in early instars for longer periods and are likely to be parasitized more than those feeding on susceptible plants (Danks et al. 1979). However, changes in host suitability due to the insect host's diet are also known to influence the developmental rate, size, sex ratio, fecundity, and life span of *C. sonorensis* (Vinson and Barbosa 1987). The present studies indicated that the size of *H. armigera* larvae had a significant influence on the development period, and size and survival of *C. chlorideae*. Although, the *Helicoverpa*-resistant chickpea genotype ICC 506 reduced the size of the parasitoid cocoons as compared to susceptible genotype L 550, there was no significant effect of chickpea genotypes on larval and pupal periods, and survival of the parasitoid, suggesting that the *Helicoverpa*-resistant chickpea genotypes are compatible with the larval parasitoid, *C. chlorideae*. The developmental period of *C. chlorideae* was prolonged, and cocoon formation and adult emergence reduced in *H. armigera* fed on *Bt*-treated chickpeas as compared to those fed on untreated controls. Therefore, growing *Helicoverpa*-resistant chickpea, augmentation of *C. chlorideae* population at the vegetative growth stage (when the activity of this parasitoid is at maximum), followed by *Bt* sprays at the reproductive stage, could be sustainable option for the management of pod borer, *H. armigera* in chickpea.

Interaction of transgenic plants with non-target insects and natural enemies has been studied by several workers (Wilson et al. 1992; Fitt 2003; Romeis et al. 2006; Sharma et al. 2007; Dhillon and Sharma 2009a). Of the non-target insects, generalist predators may be relatively less affected by the transgenic plants as they tend to feed on prey, which may or may not imbibe the transgene product from the prey. On the other hand, host-specific endoparasitoids are likely to get exposed to the transgene product through insect host feeding on the transgenic plants (Vojtech et al. 2005; Ramirez-Romero et al. 2008). However, no adverse effects of transgenic maize have been observed in case of *Eriborus terebrans* (Gravenhorst) and *Macrocentrus grandii* (Goidanich) parasitizing European corn borer, *Ostrinia nubilalis* (Hubner) (Orr and Landis 1997). *Campoletis sonorensis* and transgenic plants also act synergistically and decrease survival of *H. virescens* larvae beyond the level expected of an additive interaction (Johnson and Gould 1992). *Helicoverpa*-resistant chickpeas showed no adverse effects on the survival and development of the host-specific parasitoid, *C. chlorideae*. However, the developmental period of *C. chlorideae* was prolonged and survival reduced when the insect host, *H. armigera* larvae were fed on *Bt*-treated chickpeas. This prolonged developmental period might be because of poor nutritional quality of the host larvaeand, reduced parasitoid survival due to early mortality of *H. armigera* larvae. Reduced cocoon formation and adult emergence, and prolonged larval period of *C. chlorideae* have been observed on *H. armigera* larvae fed on artificial diets containing the *Bt* toxins Cry1Ab and Cry1Ac (Sharma et al. 2008). Sublethal doses of *Bt* toxins may also reduce the nutritional quality of the insect host, which has been shown to result in negative effects on the development and survival of some natural enemies (Nordlund et al. 1988; Murugan et al. 2000). Cry1Ab and Cry2A intoxicated larvae of *Spodoptera littoralis* (Boisduval) result in same adverse effects on *Chrysoperla carnea* (Stephens) (Hilbeck et al. 1999; Dutton et al. 2002, 2003). Ingestion of *Bt* toxins decreases the concentrations of essential amino acids such as isoleucine, leucine, methionine, threonine, and valine in the haemolymph of *S. littoralis* (Salama et al. 1983), and these amino acids are also important for the development of *C. carnea* larvae (Yazlovetzky 2001). Decrease in amounts of some essential amino acids might be one of the possible mechanisms by which *Bt*-intoxicated *H. armigera* larvae might have affected the parasitoid, *C. chlorideae*.

The exposure of *C. chlorideae* adults to *Bt* toxins in 10% honey reduced adult longevity and fecundity, which might be because of exposure to other *Bt* toxins/spores than to Cry1Ab or Cry1Ac or poor feeding due to other ingredients present in the *Bt* formulation, as no adverse effects of *Bt* were observed on the progenies. Although *H. armigera* fed on chickpea plants treated with *Bt* showed some negative effects on fitness and survival of *C. chlorideae*, these effects were largely indirect and host mediated (poor quality or early mortality of the host larvae), since no *Bt* toxin protein was detected in any of the life stages of *C. chlorideae* reared on *H. armigera* larvae fed on *Bt*-sprayed chickpeas, and the adults fed on *Bt*-contaminated honey. In addition, there were no carry-over adverse effects of *Bt* on the development, survival and progeny production of *C. chlorideae* in the following generation. Moreover, *C. chlorideae* exposure to *Bt* through *Bt*-treated chickpea-fed *H. armigera* larvae resulted in increased cocoon formation, adult emergence, and the fecundity in the following generation, which might be because of selection of the most vigorous parasitoids from the *Bt* exposed generation. These studies have generated useful information on compatibility of *H. armigera*-resistant chickpea genotypes, *Bt* sprays, and the parasitoid, *C. chlorideae per se*, and the protocols for testing non-target effects of *Bt*-transgenic chickpeas on natural enemies for developing appropriate strategies for deployment of *Bt*-transgenic chickpeas for controlling *H. armigera*.

Acknowledgements

The technical support of ICRISAT entomology staff during the studies, funding by Swiss Agency for Development and Cooperation (SDC), Berne, Switzerland, and the Department of Biotechnology (DBT), New Delhi, India, and IndoSwiss Collaboration on Biotechnology (ISCB), and the helpful comments by two anonymous referees on the manuscript are gratefully acknowledged.

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