Interaction between Bt-transgenic cotton and the whitefly's parasitoid, *Encarsia formosa* (Hymenoptera: Aphelinidae)

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Abstract: Transgenic Bt cotton developed against lepidopteran pests may not be compatible with parasitoid of secondary pests such as *Bemisia tabaci* which attack many plants such as cotton. In this study, the effects of Bt cotton on the demographic parameters of *Encarsia formosa*, parasitoid of *B. tabaci* were assessed. The data were analysed using the age specific, two-sex life table parameters. The results indicated that pre-adult developmental time, the total preoviposition period (TPOP) and the adult preoviposition period (APOP) in the Bt cotton were significantly longer than in the non-Bt cotton. Also, fecundity and body size in both lines were significantly different. The fecundity was 23.64±0.73 and 43.75±0.89 eggs/females in the Bt and non-Bt cotton, respectively. All the population parameters were affected by the Bt cotton. The intrinsic rate of increase (r) was 0.15 day^{-1} in the non-Bt cotton but it was 0.10 day^{-1} in the Bt cotton. The finite rate of increase (λ) was 1.11 day^{-1} in the non-Bt cotton whilst it was 1.08 in the Bt cotton. The net reproductive rate (R_0) in the non-Bt cotton was 36.75 but in the Bt cotton these parameters showed 19.62 offspring/individual. Also, the mean generation time (T) in the non-Bt and Bt cotton was 22.69 and 27.79 days, respectively. The results illustrated, that although transgenic crops are effective tools for management of the target pests, they can adversely affect, either directly or indirectly, the natural enemies dependent on these plants.

Key words: Aphelinid parasitoid, biological control, demographic parameters, non-target organism, transgenic crop

Introduction

One of the most important principles of the Integrated Pest Management programs (IPM) is use of non-chemical agents such as natural enemies (parasitoids and predators), and resistant host plant, to control pests. The compatibility of such factors in IPM has been tested in several projects (Pedigo and Rice 2009). Production of transgenic crops was a novelty in the method of applying host-plant resistance. Crops that are genetically engineered to express *Bacillus thuringiensis* toxin are planted in many areas. Such crops are growing in number each year and covered over 58 mln ha globally, in 2010. Maize is the most widely cultivated Bt crop (James 2010).

Considerable efforts have been done to determine the effects of Bt crops on non-target arthropods (Hilbeck *et al.* 1998; Ashouri *et al.* 2001; Raps *et al.* 2001; Ashouri 2004; Burgio *et al.* 2007; Faria *et al.* 2007; Mellet and Shoeman 2007; Lawo *et al.* 2009). Up till now, though, little has been known about the effects on natural enemies (Hilbeck and Schmidt 2006). Host plants in a tritrophic system including that of the plant-herbivore-natural enemies, can have an effect on the fitness of natural enemies due to the qual-

ity and quantity of the herbivore preys (Price *et al.* 1980). Although the resistant crops can remain safe against the herbivores, natural enemies may be affected adversely. This matter is more highlighted in the parasitoids because they are more dependent on the host quality and abundance. Therefore, cultivation of transgenic plants, potentially has effects on the parasitoid through herbivore quality (indirect effect). Also, when they feed on the nectar and pollen of transgenic plants or during the sap sucking of honey dew, they are exposed to transgenic plants (direct effects) (Greenplate 1997, 1999). Previous studies illustrate that natural enemies can also be exposed to the proteins expressed in the transgenic plants (directly by feeding on transgenic plant nectar or indirectly through the feeding of their host organisms living on the transgenic plants) (Arpaia *et al.* 2009). Parasitoids appeared to be more sensitive than predators to diets containing Cry toxins (Lövei *et al.* 2009). Negative effects occurred when parasitoids were feeding on hosts sensitive to the toxins expressed in the transgenic plants. Most of the indirect effects are not shown in the published results. However,

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it seems that host quality has more effects on the biology of parasitoids (Naranjo 2009).

The effect of plant resistance at the third thyrotrophic level is difficult to predict because of the wide variability of plant resistance mechanisms (Ashouri *et al*. 2001). Thus, predicting plant interaction with the natural enemies in the transgenic plants may be difficult (Ashouri *et al*. 2001). Due to the reduced use of chemical pesticides in transgenic fields, secondary pests may be controlled sufficiently by their natural enemies. The sweet potato whitefly, *Bemisia tabaci* (Gennadius), is a polyphagous insect attacking many plant species of economic importance, such as vegetables, oil, and ornamental plants (Oliveira *et al*. 2001). The sweet potato whitefly transfers more than 100 plant viruses (Cock 1993). Although *B. tabaci* is a key pest, it plays an important role in the ecosystem and food chain since its honeydew is a food source for many arthropods. By reducing pesticide application, natural enemies can play an important role in the control of *B. tabaci* in the Bt cotton fields. Biological control of this insect pest is frequently based on the use of the Aphelinid parasitoid, especially the genera of *Encarsia*.

Encarsia formosa (Gahan) (Hymenoptera: Aphelinidae) is probably the most worldwide used parasitoid for control of *Trialeuroides vaporariorum* (Westwood) and *Bemisia* spp. in greenhouses (Hoddle *et al*. 1998). The male is rare in the parasitoid and the female does not need courtship for fertilisation. The female prefers to lay her eggs into the third or early fourth instar nymph body of the greenhouse whitefly. Adult *Encarsia* feed on honeydew and on the body content of first and second instar whitefly nymphs (Boisclair *et al*. 1990).

According to Southwood (1966), the "intrinsic rate of increase", the most important parameter of life table parameter, is used to compare the population growth potential of different species affected by food quality. This study was conducted to evaluate the effects of host quality (*B. tabaci* reared on the Bt cotton) on the growth and development of *E. formosa*. So far, there are few studies about the adverse effects of Bt cotton on natural enemies such as *E. formosa*. In this study, for the first time, the effects of the Bt cotton on the life table parameters of *E. formosa* were investigated. In addition to the life table history, a comparison was also done on the body weight, and sexual ratio of the parasitoid, of Bt cotton and non-Bt cotton treatments.

Materials and Methods

Cultures

The Bt cotton Line 61, produced in Iran for the first time (Tohidfar *et al*. 2008), was taken from the Institute of Agricultural Biotechnology. The non-Bt cotton was obtained from the Institute of Agricultural Biotechnology. Both lines were planted into pots placed in net covered cages (75 × 75 × 75 cm) and kept in a greenhouse under climate controlled conditions at 27±1°C, 60% RH, and 16 : 8 h (light : dark). Plants with 4–8 leaves were used for the experiment. The whiteflies, *B. tabaci* (Homoptera: Alyrodidae) originally were collected from the cotton field

in Gorgan city, Iran. The whiteflies were reared on the Bt and non-Bt cotton plants, for four generations, in cages at the same condition. *E. formosa* was collected from tomato fields in Hashtgerd, the Alborz province of Iran, and reared for four generation on the *B. tabaci* in the above conditions.

Life table study of *E. formosa* on Bt and non-Bt cotton

Third instar nympha (120 individuals) of *B. tabaci* that had developed on both of the cottons (Bt cotton and non-Bt cotton) were individually exposed to a single attack (0–24 h hold) by *E. formosa* that were held in an individual cage with 10% sugar water. After 24 h, the whiteflies were returned to a host plant of the same line under these conditions: 23±4°C, 65±10% RH and a 16 : 8 h (light : dark) photoregime, until emergence of an adult parasitoid.

After 5 days (expected delay for egg eclosion at a minimum temperature of 19°C), 10 presumably parasitised whiteflies per plant (one whitefly per Bt cotton and non-Bt cotton) were randomly selected for dissection to determine egg eclosion incidence. When whiteflies turned black, indicating parasitoid survival to the pupal stage had started, checking frequency was increased to three per day. Newly black pupa were placed in individual gelatin capsules. This allowed determination of emergence success, development time from egg to pupation and from pupation to emergence.

Developmental time of all individuals (egg + larva), pupa, and adults – including males (rarely observed in the parasitoid), females and those dying before the adult stage, and female daily fecundity were analysed according to the age-stage, two-sex life table theory (Chi and Liu 1985; Chi 1988). The following population parameters of each cohort were estimated:

– reproductive rate (R_0):

$$R_0 = \sum_{x=0}^{\infty} l_x m_x,$$

– intrinsic rate of increase (r):

$$\sum_{x=0}^{\infty} e^{-1(x+1)} l_x m_x = 1,$$

– mean generation time (T):

$$T = \frac{\ln(R_0)}{r},$$

– finite rate of increase (λ):

$$\lambda = e^r,$$

where $l_x m_x$ is age specific maternity.

Data analysis and population parameters (r, R_0, T, gross reproduction rate – GRR, and λ) were estimated using the TWOSEX-MSChart program (Chi 2013). The TWOSEX-MSChart is available at http://140.120.197.173/Ecology/prod02.htm (Chung Hsing University). The means

and standard errors of the life table parameters were estimated using the bootstrap techniques (Efron and Tibshirani 1993) embedded in the TWOSEX-MSChart (Chi 2013). Survival, fecundity, and reproductive value curves were constructed using SigmaPlot 11.0.

To determine adult dry weight, newly emerged parasitoids were placed in an oven at 60°C for 48 h and weighed using a microbalance (sensitivity 0.001 mg). We used the Student t-test to determine the differences between the results of the population parameters of two treatments (Zar 1999).

Results and Discussion

Pre-adult developmental time (egg + larva + pupa) of *E. formosa* on the Bt cotton and non-Bt cotton was 23.64 and 17.82 days, respectively. Thus, developmental time in the Bt cotton was significantly longer than in the non-Bt cotton (Table 1). In fact, the pre-adult developmental rate was significantly (t = 12.31, p < 0.0001) affected by the Bt cotton.

The total preoviposition period (TPOP) and the adult preoviposition period (APOP) were significantly affected by the Bt cotton (Table 1). On the non-Bt cotton, *E. formosa* reached the adult stage faster and started to lay eggs sooner than *E. formosa* on Bt cotton. The amount of eggs per female in the non-Bt cotton was also significantly more than the Bt cotton (Table 1).

Out of the 100 eggs initially selected for the life table study in Bt cotton and non-Bt cotton, 84 and 83 eggs emerged as adults, respectively. The death number of parasitoids during the preadult stages in both the Bt cotton (15) and non-Bt cotton (17) was low. The Bt cotton did not have any significant negative effects on the survival of *E. formosa*.

The life table parameters are shown in table 2. Statistical analysis demonstrated that there were significant differences in all the parameters (p < 0.05). The following parameters: r, λ, and R_0 in the Bt cotton were less than for the non-Bt cotton. However, T in the Bt cotton was longer than that in the control. The higher r was due to the lower developmental time and earlier oviposition of *E. formosa* in the control.

The results showed the weight of the parasitoids at the time of emergence was markedly affected by the Bt cotton. The body weight of *E. formosa* on the Bt cotton (11.8±0.98 μg) was significantly less (t = 6.67, p < 0.0001) than that on the non-Bt cotton (19.28±0.28 μg).

Several studies (Baur and Boethel 2003; Liu *et al*. 2005a, b) have previously shown the negative interaction between host plant resistance and some hymenopteran parasitoids. Such results emphasise the probability of Bt cotton having negative effects on some beneficial organisms like predators and parasitoids. Several numbers of plant factors such as plant species and morphological features can affect the efficiency of *E. formosa* (Hoddle *et al*. 1998). In fact, food quality is one of the main factors in developmental longevity, body size, and fecundity abundance (Hódar *et al*. 2002). Faria *et al*. (2007) showed that Bt corn lines (*Zea mays* Linné) in comparison with non-Bt lines, generally induced lower concentrations of leucine and isoleucine. According to Yan *et al*. (2004), Bt genes inserted into plants induce changes in secondary compounds that are extremely important for herbivore/cotton plant interactions. It also seems that overexpression of the toxin in the foliage, reduces free amino acids available in phloem sap (which poses as nutritional value for whiteflies) (Burgio *et al*. 2011). According to the perspective of interaction between plant resistance and biological control agents, the current results were similar to those

Table 1. Life history statics (mean ±SE) of *E. formosa* in Bt and the control cotton at 27°C

Parameters	Egg + Larva [days]	Pupa [days]	Total pre-adult [days]	APOP[a] [days]	TPOP[b] [days]	Fecundity [eggs/females]	Body size [μg]
Non-Bt cotton	11.22±0.28	6.6±0.13	17.94±0.31	0.05±0.5	0.26±18.49	0.89±43.75	19.28±0.28
Bt cotton	13.35±0.29	10.29±0.09	23.75±0.35	0.23±0.04	0.34±24.02	0.73±23.64	11.8±0.98
t-Student	5.36	5.31	12.31	3.4	11.72	14.06	t = 6.67
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
df	179	173	165	163	165	167	5,998

Means in a column followed by different letters are significantly different (p < 0.05) (t-test)
[a] adult preoviposition period; [b] total preoviposition period

Table 2. Life table parameters (mean ±SE) of *E. formosa* on Bt cotton and the control cotton at 27°C

Parameters	r [day⁻¹]	λ [day⁻¹]	R_0 [offspring/individual]	T [days]
Non-Bt cotton	0.15xx±0.00yy	1.11xx±0.0048	36.75±2.25	22.69±0.28
Bt cotton	0.10±0.0031	1.0814±0.0034	19.62±1.62	27.79±0.34
t-Student	1,326.01	1,318.0	828.24	828.24
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001
df	19,998	19,998	19,998	19,998

r – intrinsic rate of increase; λ – finite rate of increase; R_0 – reproductive rate; T – mean generation time

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authors (Hilbeck *et al*. 1998; Ashouri *et al*. 2001; Guo *et al*. 2004; Burgio *et al*. 2011) who found that plants expressing Bt toxin, negatively affected the natural enemies.

Bt plant toxins may be sequestered in the haemolymph or body tissues of herbivores. The result is an indirect effect on the natural enemies seen through such factors as delayed development, reduced hatching rates, and low performance. The meta-analysis conducted by Naranjo (2009), confirmed the higher sensitivity of parasitoids to Cry toxins. Ramirez-Romero *et al*. (2007) observed that exposure to Cry1Ab toxin via hosts fed on Bt maize tissues, sublethally affected the parasitoid wasp *Cotesia marginiventris* (Cresson).

Our results made clear that developmental time of the parasitoids increased in Bt cotton during pre-adult stages. However, increased developmental time in the field may not necessarily lead to increased parasitism because of reduced host density, as Hagerty *et al*. (2000) indicated. On the other hand, *E. formosa* survival did not change considerably. Since the host quality became poor, the other ecological parameters important in parasitism (especially during the next generations) such as fecundity were affected adversely.

In this study *r* in the *E. formosa* rearing on the Bt cotton was less than that on the non-Bt cotton. This indicated that *E. formosa* grows slower on Bt cotton. The relatively poor host attribute of Bt cotton for *E. formosa*, caused delays in the development. The Bt cotton has different characteristics that affect the life table parameters (T, R_0, r, λ) of the parasitoid.

The age-stage survival rate (s_{xj}) shows the probability that a newborn egg would survive to age x and stage j (Fig. 1). Significant overlapping among different stages can be seen because of the variable developmental rates among individuals that were accounted for in the age-stage, two-sex life table (Chi and Yang 2003). In this study, emergence of the female adults on non Bt cotton started at day 11 (4 days earlier than that on Bt cotton). Also maximum females alive on non Bt cotton was seen on day 21 and the maximum females alive was 8 days earlier than that on Bt cotton.

Different studies on the predators and parasitoids demonstrated the higher mortality of these organisms after feeding on non-target herbivores reared on the Bt crops (Hefaz *et al*. 1997; Chenot and Raffa 1998; Hilbeck *et al*. 1998a, b). But in this research study, the survival of
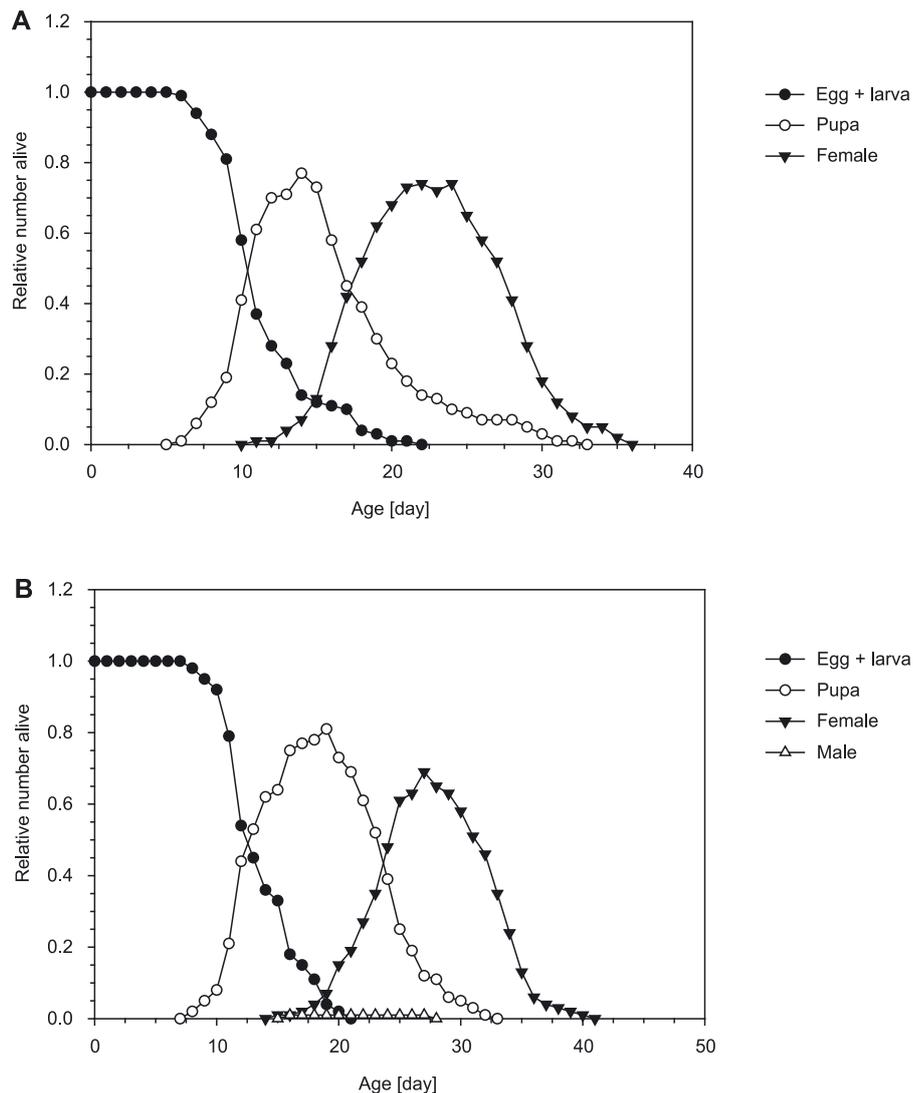

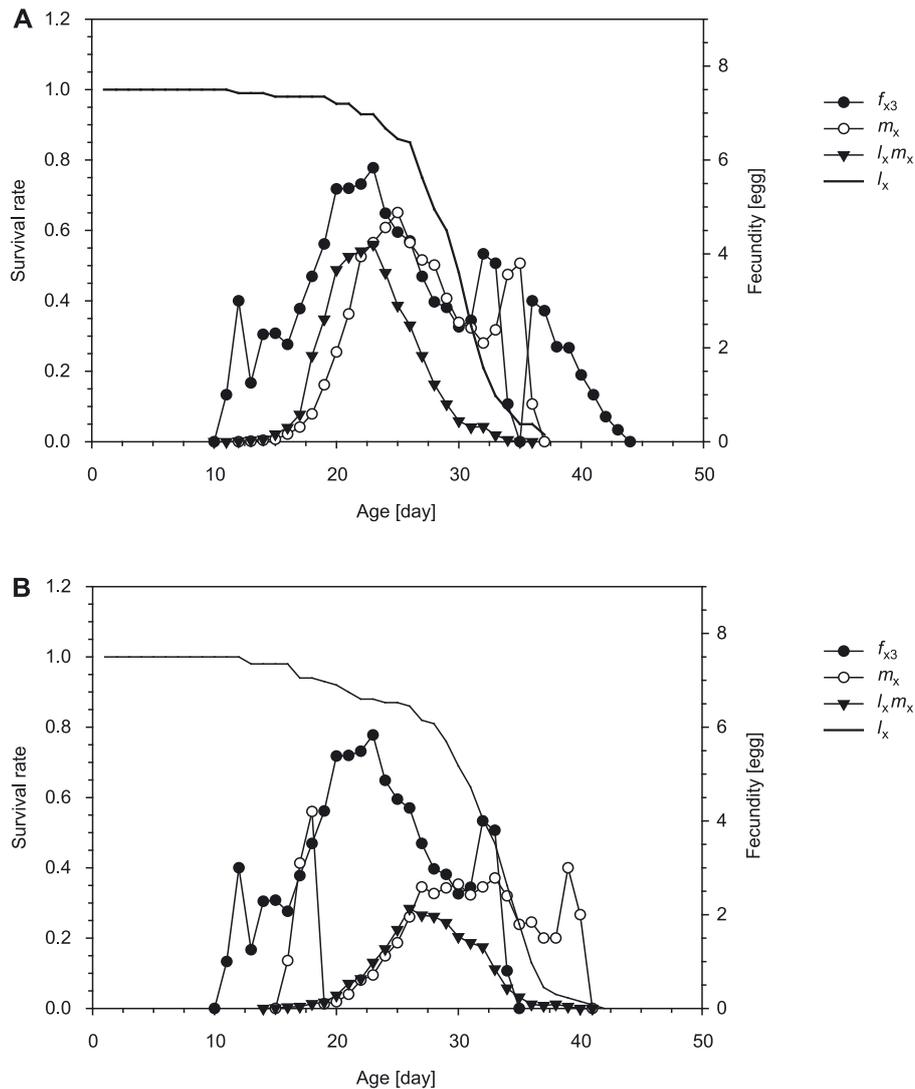**Fig. 1.** The age-stage survival rate (s_{xj}) of *E. formosa* in non-Bt cotton (A) and Bt cotton (B)

Fig. 2. Age-specific survival rate (l_x), female age-stage specific fecundity (f_{x3}), age-specific fecundity (m_x), and age specific maternity ($l_x m_x$) of *E. formosa* in non-Bt cotton (A) and Bt cotton (B)

E. formosa was not affected by the Bt cotton, and this crop did not have any antibiotic effects on this parasitoid. Thus, the demographic parameters of s_{xj} did not change after rearing individuals on Bt cotton. This statistic (s_{xj}) gives a detailed description of the survivorship as well as stage transitions. The age-specific survival rate calculated according to Chi and Liu (1985) is defined as:

$$l_x = \sum_{j=1}^{\beta} s_{xj},$$

where β is the number of stages.

In fact, l_x is a simplified form of s_{xj} and shows how survivorship decreased with age. As shown in the figure 2, l_x had an almost similar trend in both treatments (Bt and non-Bt cotton). However, age-specific fecundity of the total population (m_x) shows a sharp decline in Bt cotton for all of the days. As a result $l_x m_x$ in non-Bt cotton is calculated higher than that on Bt cotton.

Values for l_x of total cohort, female age-stage specific fecundity (f_{x3}), m_x, and $l_x m_x$ are presented in figure 2. Since only females produce eggs, there is only a single curve (f_{x3}) that represents females in the third life stage.

A reduction has been seen in the fitness of *E. formosa* on Bt cotton. The results were similar to the outcomes showing that antibiotic plant resistance generally reduces herbivore fitness, such as size (Ashouri *et al.* 2001). In fact, the parasitoids may be adversely affected by their hosts whose quality, such as growth rate was reduced due to being reared on the Bt-crops expressing high levels of toxin. As a result, a decrease could be measured in the parasitoid size and fecundity (van Emden 1991).

In addition, Ashouri *et al.* (2001) confirmed that different lines of potato resistance have various effects on parasitoid fitness. Also Ashouri *et al.* (2001) reported that the small size of the adult aphids was due to feeding on transgenic crops, consequently bringing about delays in reproduction, and a decrease in adult aphid numbers. The side effects of transgenic Bt potatoes on smaller insects can lead to a reduction in survivorship, developmental time, and the intrinsic rate of increase.

The variation of *E. formosa* adult weight with the cotton line correlates with the weight variation of the whitefly's hosts on these lines (Azimi *et al.*, unpublished data).

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The weight of adult individuals of *B. tabaci* on the Bt cotton was lower than the weight of those in the control. The size of the whiteflies is a key factor for the parasitoid fitness because parasitoid fitness depends on host quality (Ashouri *et al.* 2001).

Conclusions

The use of Bt cotton leads to suppression of its lepidopteran target pests and reduction in pesticides applications. The reduction in pesticide applications can conserve hymenopteran parasitoid populations. *E. formosa* is an important hymenopteran parasitoid of whiteflies. The whitefly does not seem like a target for Bt toxins. Nevertheless, as shown in this study, they could be affected by the transgenic cotton. Several ecological parameters of the parasitoid such as growth and development were negatively affected by the Bt cotton, indirectly. It is important to note, though, that the present study was done in constant laboratory conditions and it was hard to predict the fate of a natural enemy in a transgenic crop field where some other characteristics such as climate factors could be more important than cultivar effect (Górecka *et al.* 2008; Twardowski *et al.* 2012). Nevertheless, it is necessary to recognise the effects of transgenic plants on non-target organisms such as *E. formosa* especially when the transgenic crops are used in IPM.

Acknowledgments

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