

Article

Effect of Iranian Bt cotton on life table of *Bemisia tabaci* (Hemiptera: Alyrodidae) and Cry 1Ab detection in the whitefly honeydew

Solmaz Azimi¹, Shima Rahmani², Masoud Tohidfar³, Ahmad Ashouri⁴

¹Department of Plant Protection, Azarbaijan Shahid Madani University, Tabriz, Iran

²Department of Entomology, Science and Research Branch, Islamic Azad University, Tehran, Iran

³Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran

⁴Department of Plant Protection, Faculty of Sciences & Agricultural Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

E-mail: s_azimi2007@yahoo.com

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Abstract

Transgenic cotton expressing the Cry 1Ab protein of *Bacillus thuringiensis* developing against *Helicoverpa armigera* may be affect on secondary pest such as *Bemisia tabaci*. In this study effects of Bt cotton on demographic parameters of *B. tabaci* were assessed and the data analyzed using the age specific, two-sex life table parameters. Results showed that getting to the adulthood stage, was faster on non-Bt cotton in comparison with Bt cotton. Also the fecundity was higher on non-Bt cotton than that on Bt cotton. Some of the population parameters (r , R_0 and T) of *B. tabaci* were affected by the Bt cotton significantly. The intrinsic rate of increase (r) on Bt and non-Bt cotton was 0.07 day^{-1} and 0.1 day^{-1} , respectively. The net reproductive rate (R_0) was 20.68 and 15.04 offspring/individual on Bt and non-Bt cotton, respectively. Mean generation time (T) in non-Bt cotton was 27.22 and 34.62 days in Bt cotton. The results indicated that the life history of *B. tabaci* in the laboratory condition was influenced by host plant quality and Bt cotton was not a suitable host for *B. tabaci*. The western immunoblot method showed that the Cry protein detection in honeydew was positive which indicated that the Cry protein was ingested. Results revealed that the transgenic cotton could adversely affect the secondary pest and the natural enemies which feed on such pests as a host or their honeydew as a food source should be considered.

Keywords Bt cotton; demography; *Bemisia tabaci*; transgenic plant.

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1 Introduction

Today transgenic crops are planted throughout the millions hectares of the world. Bt cotton is a transgenic plant made by transition of the *cry* gene from *B. thuringiensis* to the cotton. *B. thuringiensis* is a gram

negative soil bacterium that produces Cry insecticide proteins (Arparia, 2009). Cry1 expressed into the cotton, protects the plants against Lepidopteran pest damages and shows high level of resistance to the primary pests especially *H. armigera*.

Although the mode of action of the Cry toxins in the susceptible target insects especially lepidopteran and coleopteran pests are well studied, several details are still not understood well. Thus, there are different explanations about Bt mode of action in midgut of target pests (Zhang, 2006; Ckrickmore, 2005; Broderick et al., 2006). Considering the fast increasing the global production area of Bt crops, it seems need to understand how Bt toxins might affect non-target organisms (Hilbeck and Schimdt, 2006).

In order to study the effects of transgenic plants should not be only limited to target insects but it is necessary to have a better knowledge about the unexpected effects on non-target pests. Entomologists hope that studies about the effect of Bt plants on non-target organism offers great opportunities to improve our knowledge about the Bt function. They explain that Bt crops have direct and indirect effects on non-target pests (Hilbeck and Schimdt, 2006). Non-target pests or secondary pests for example in cotton fields are aphids and whiteflies. Direct effects on non-target pests are not well known and probably including of antibiotic effects. On the other hand, Bt crops can affect indirectly on non-target pests by changing the plant host quality and reduction in fitness (developmental time, fecundity, etc.) of non-target pests living on the Bt crops. Attraction of Bt crops for the diverse pests is different (Hilbeck and Schimdt, 2006). In an experiment, Bt cotton cultivars NuOPAL and FM 975 were used to determine biological parameters of *Bemisia tabaci* (Gennadius), as a non-target pest of Bt crops. Both Bt cotton cultivars caused reduction in approximately 20 percent of adult emergence of the whitefly (Pessoa et al., 2016). Also, Chandi and Kular (2014) studied biological parameters of *Bemisia tabaci* on different cotton cultivars and illustrated differences between these cultivars and non-Bt cotton.

Bemisia tabaci as a secondary pest of cotton plays an important role in the ecosystem and food chain. In fact, its honeydew is a food source for many arthropods. In addition to *B. tabaci* itself is used as a host or prey for many parasitoids and predators. However this insect pest transfers more than 100 plant viruses (Cock et al., 1993). Evaluation of the Bt cotton effect on *B. tabaci* and detection of Bt protein in the honeydew, help us to reply most of the questions about the Bt crops impact on non-target insects and food chains. *B. tabaci* may not be affected by feeding on Bt cotton but accumulation of the Bt toxin in its body, can affect its predators or parasitoids negatively (Hilbeck and Schmidt 2006).

Effects of Bt plants on secondary pests especially aphids were estimated, ranging from minor negative effects on the aphid survivorship and fecundity (Ashouri, 2004; Ashouri et al., 2001; Burgio et al., 2007; Faria et al., 2007; Lawo et al., 2009; Mellet and Shoeman, 2007; Raps et al., 2001). There is a little information about the Bt plants effects on *B. tabaci*. In fact, genetically differences among non-target species and/or various amount of Bt toxin protein in the transgenic crops, can be ended to different sensitivity and various responses to Bt toxin.

Southwood (1966) explained that the life table parameters such as intrinsic rate of increase, are useful indices for comparing the population growth potential of different species under different situations such as food quality. So this study was conducted to evaluate the effects of host quality on growth and development of *Bemisia tabaci* feeding on Bt cotton. We used life table history, body weight and sexual ratio to compare Bt cotton with non-Bt cotton.

2 Materials and Methods

2.1 Insect culture

The Bt cotton resistant to *Helicoverpa armigera* was produced in Iran for the first time in the Institute of Agricultural Biotechnology (Tohidfar et al., 2008). The Bt cotton Line 61 was used as experimental material

and non-transgenic cotton used as control. The crops were planted in the pots were placed in the net covered cages (75cm×75cm×75cm) in a controlled greenhouse conditions at temperature of 27±1 °C, 60% relative humidity, 16 h light: 8 h dark photoperiod. Plants with 6 leaves were used for experiment. The whiteflies, *Bemisia tabaci* (Homoptera: Alyrodidae) originally were collected from cotton fields of Golestan Province, Gorgan city, Iran, and were reared on cotton plants of both Bt cotton and non-Bt cotton for four generations in the cages and conditions was explained above.

2.2 Bt protein detection

Western-blot analyses were used for detection of Cry 1Ab proteins in the plant and honeydew of whiteflies (Tohidfar *et al*, 2008). The leaves of Bt cotton and non-Bt cotton plants during the three different stages (stage 1: appearance of cotyledon leaf, stage 2: appearance of fourth true-leaf and stage 3: flowering) were grounded to fine powder with a mortar and pestle into liquid nitrogen. Soluble proteins were extracted with 1 ml of extraction buffer [40% (w/v), SDS (Sodium dodecyl sulfate), 5% (v/v) 2-mercaptoethanol, 20% (v/v) glycerol, 68 mM Tris-HCl (pH 6.8)]. 10 µg of protein from each sample was fractionated by 13% (w/v) SDS polyacrylamide gel electrophoresis, as described by Laemmli (1970).

Protein detecting was probed with the anti-Cry1Ab anti-serum (1:30000 v/v). The goat-anti rabbit IgG alkaline phosphatase conjugate (1:2000 v/v) (Gibco, USA) was used as a secondary antibody. In order to collect the honeydew 40 plants for each Bt cotton and non-Bt cotton in 6-leaves stage were selected and leaves were arranged with a clip cage. In each clip cage, about 100-200 insects were present. After 10 days, the honeydew was collected and dissolved in extraction buffer, then centrifuged in 13000 xg for 15 min at 4°C. supernatant was transferred in to a new tube.

2.3 Life table study of *B. tabaci* on Bt and non-Bt cotton

100 eggs of *Bemisia tabaci* were collected randomly from Bt and non-Bt cotton and numbered for the next assessment. First instar nymphs slowly move and then fixed. Pupa did not have nutrition thus we kept them separately in micro-capsules till adults emergence. Larval mortality and development were checked every 12-h until the adult stage. After the emergence of adults, males and females were paired and checked daily to record survival and number of eggs laid until females dead.

Developmental time of all individuals, including males, females, and those dying before the adult stage, as well as the female daily fecundity were analyzed according to the age-stage, two-sex life table (Chi and Liu, 1985; Chi, 1988) using the computer program TWSEX-MSChart (Chi, 2014)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

$$\lambda = e^r$$

Data analysis and population parameters (r , R_0 , T , GRR and λ) were calculated using the TWOSEX-MSChart program (Chi 2013). The TWOSEX-MSChart is available in <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 values curves were constructed using SigmaPlot 11.0.

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

Newly emerged whiteflies were placed in an oven at 60 °C for 48 h to determine adult dry weight using a microbalance (sensitivity of 0.001 mg). To determine the sex ratio of *B. tabaci* offspring, 60 eggs or more were collected from females of different ages every third days after the beginning of oviposition. Totally, the egg samples were collected for 13 times. Also the hatch rate and sex ratio of these eggs were recorded. The *t*-student test was used in order to compare the means of the treatments.

3 Results

The size of the Cry1Ab protein accumulating in the leaves of T₁, T₂ and T₃ Bt cotton was about 67 KDa (Fig. 1). Untransformed non-Bt cotton (line 5) did not show any positive signal for Cry1Ab protein. Cry was detected in Bt cottons and honeydew (line 4) and showed that the Cry protein was transferred from Bt cotton to the honeydew.

Pre-adult developmental time of this insect on the Bt cotton and non-Bt cotton was 22.48 and 20.06 days, respectively. Thus developmental time in the Bt cotton was longer than that on non-Bt cotton. Pre-adult developmental ratio was significantly ($t=23.02$, $P<0.00001$) affected by Bt cotton.

Bemisia tabaci reached to the adult stage on non-Bt cotton faster than that on Bt cotton so the females fecundity on non-Bt cotton was higher than that on Bt cotton. Results showed that total oviposition in Bt cotton was significantly ($t=8.61$, $P<0.0001$) lower than that in non-Bt cotton.

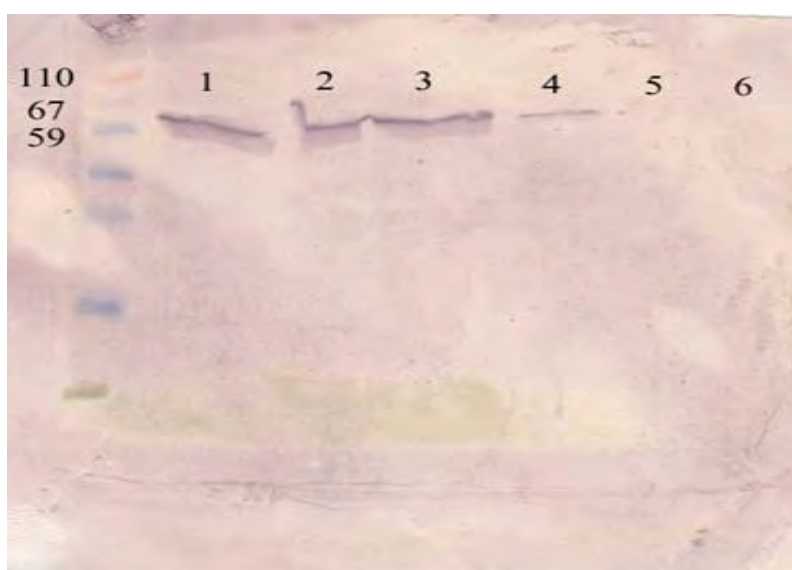


Fig. 1 Immunoblot analysis of cotton lines (1, 2, 3 and 5 non-Bt cotton) for detection of Cry protein in honeydew (4) and control honeydew (6).

The life table parameters were calculated based on data of the entire cohort, i.e., both sexes and the variable developmental rates among individuals. Calculated parameters and standard errors of the intrinsic rate of increase (r), net reproductive rate (R_0), mean generation time (T), and the finite rate of increase (λ) obtained using age-stage specific two sex model are shown in Table 1. Statistical analysis indicated that there were significant differences in r , R_0 and T between the both Bt and non Bt cotton calculating by the t-test ($P < 0.05$). The lower developmental time and earlier oviposition of *B. tabaci* in the non-Bt cotton was due to the larger intrinsic rate of increase (r). Also the mean generation time (T) in Bt cotton was longer than that in non-Bt cotton.

Table 1 Life table parameters (mean \pm SE) of *Bemisia tabaci* on the Bt cotton and the non-Bt cotton at 27°C.

| Parameters | Non-Bt cotton | Bt cotton | t-student | P |
|---------------------------------|----------------------|---------------------|-----------|---------|
| r (day ⁻¹) | 0.11129 \pm 0.0042 | 0.0783 \pm 0.0031 | 2.020 | 0.0440* |
| λ (day ⁻¹) | 1.1177 \pm 0.0048 | 1.0814 \pm 0.0034 | 1.202 | 0.2300 |
| R_0 (offspring/individual) | 20.68 \pm 2.25 | 15.04 \pm 1.62 | 2.028 | 0.0433* |
| T (day) | 27.22 \pm 0.28 | 34.62 \pm 0.34 | 9.971 | 0.0001* |

*($P < 0.05$) significance level.

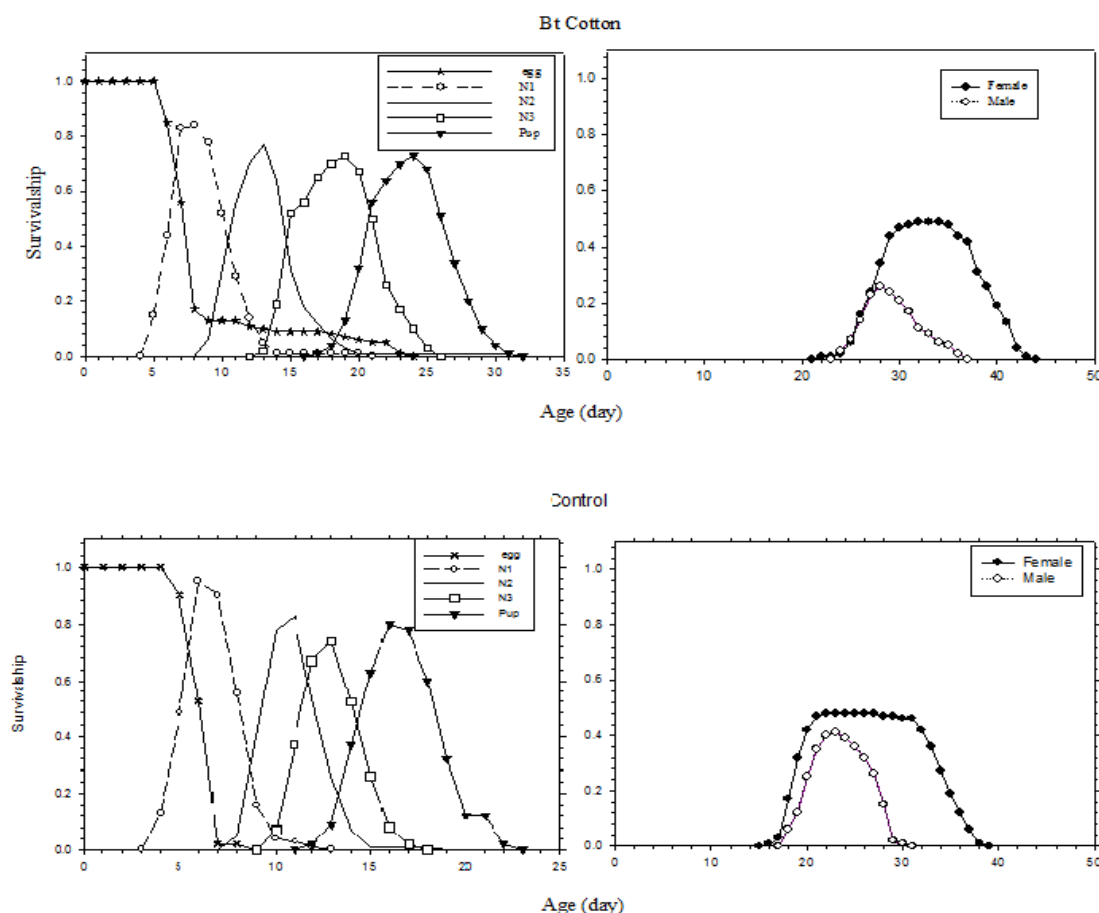


Fig. 2 Relative number of *B. tabaci* in each age-stage group s_{xj} in Bt and non-Bt cotton treated individual.

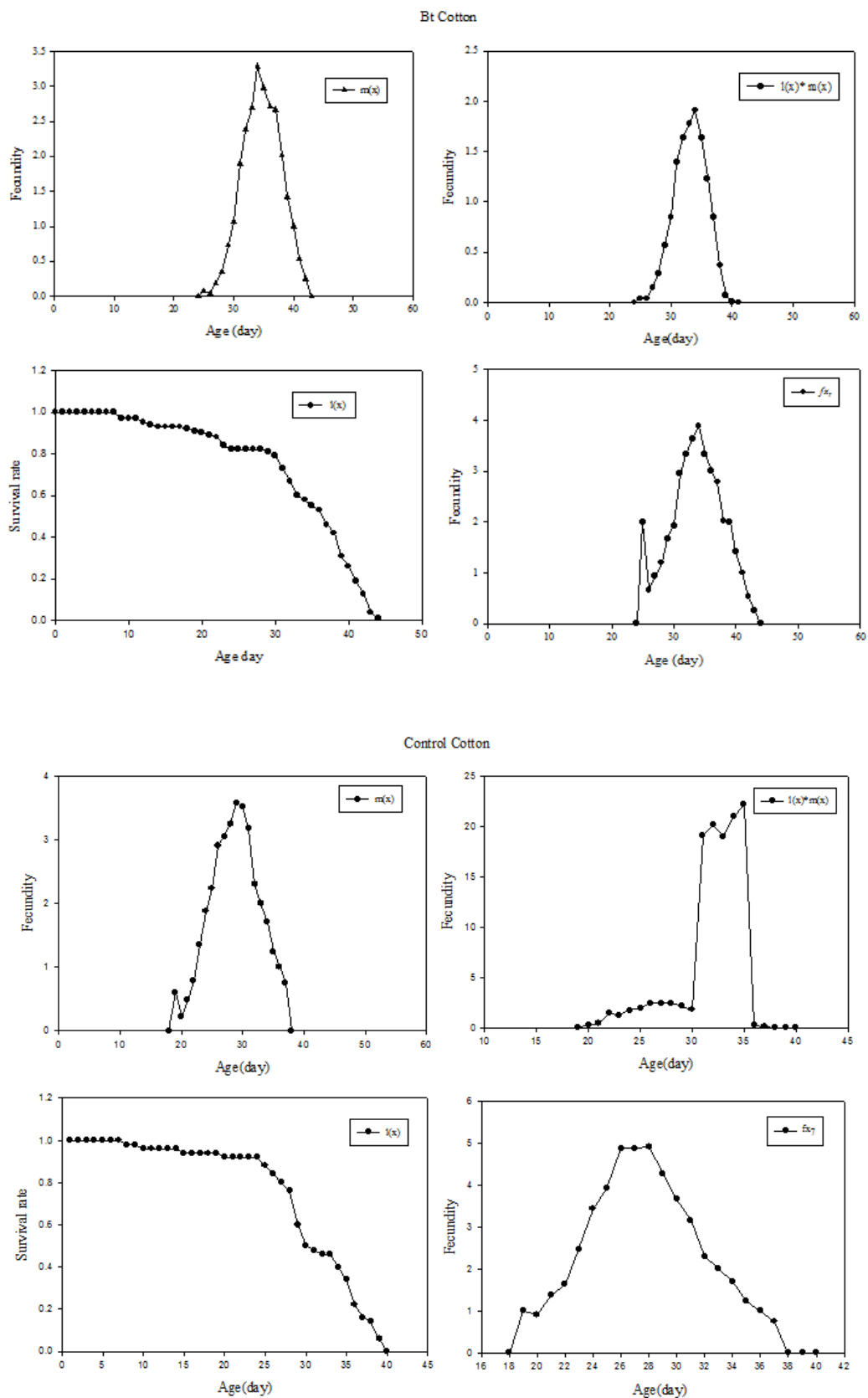


Fig. 3 Age-specific survival rate (l_x), female age stage fecundity (f_{x7}), age-specific fecundity (m_x), and age specific maternity ($l_x m_x$) of *B.tabaci* in non-Bt cotton and Bt cotton treated individuals.

For the age-stage survival rate (s_{xj}) which shows the probability that a newborn egg would survive to age x and stage j (Fig. 2), significant overlapping among different stages can be seen because of the variable developmental rates among individuals that were accounted in the age-stage, two-sex life table (Chi and Yang, 2003). Values for the age-specific survival rate of total cohort (l_x), female age-stage specific fecundity (f_{x6}), age-specific fecundity of the total population (m_x) and age specific maternity ($l_x m_x$) are presented in Fig. 3. Because only females produce eggs, there is only a single curve, f_{x6} , which represents females in the seventh life stage. l_x is a simple form of s_{xj} and shows how survivorship decreases with time.

Also results showed that the body weights of *Bemisia tabaci* on the Bt cotton ($90.8 \pm 1.98 \mu\text{g}$) was significantly lower ($t=46.67$, $P < 0.00001$) than that on the non-Bt cotton ($112.23 \pm 1.28 \mu\text{g}$). Whiteflies weight at emergence was significantly affected by the Bt cotton. Whiteflies had the smaller size after emergence on the Bt cotton while they had bigger size on the non-Bt cotton.

4 Discussion

In this study the effect of Bt cotton on *Bemisia tabaci* was investigated life table parameters of *B. tabaci*. Bt plant toxins may have indirect effect on secondary pests like *B. tabaci* by delayed development, reduced hatching rates and low performance. Smaller r (intrinsic rate of increase) in Bt cotton indicated that *B. tabaci* grows slower on the Bt cotton than non-Bt cotton. The relatively poor host attribute of Bt cotton for *B. tabaci*, caused a delay in the development. Bt cotton with different characteristics has potential to affect on life table parameters of *B. tabaci*. Although Cry protein was detected in the honeydew, the death number was not affected by the Bt cotton. As a result, Bt cotton did not have any antibiotic effect on *B. tabaci*. These outcomes were obtained by the same researchers who demonstrated that antibiotic plant resistance generally reduced fitness of herbivores such as size (Ashouri, 2004). Adult aphids with smaller size negatively affected population growth in the field through lower reproduction or delay in producing offspring. Side-effects of the transgenic-Bt potatoes for the smaller insects showed reduction in survivorship, decline in intrinsic rate of increase and slower rate of growth and development (Ashouri, 2004).

The detection of protein showed that honeydew has Cry1Ab protein. According to the previous results, the proteinous toxin reduces absorption of amino acids and glucose (Fast and Angus, 1965; Gringorten, 2001). It is possible that over expression of the *B. thuringiensis* toxin in the foliage declines the availability of aphid nutrients such as free amino acids in phloem sap (Yan et al., 2007). According to Faria et al. (2007), the lines of Bt corn (*Zea mays* Linné) generally exhibited lower concentrations of leucine and isoleucine in the sap in comparison with the non-Bt lines. Therefore, food quality can be one of the main factors in developmental longevity, body size and fecundity abundance (Hóðar et al., 2002). According to Yan et al. (2004), insertion of the Bt genes in plants, induces changes in secondary compounds that are extremely important for herbivore/cotton plant interactions. As host plant quality is widely known to have effects on insect distribution, abundance, development and fecundity, the current results obtained from *B. tabaci* rearing on the Bt cotton can be explained.

While Bt toxin is passed on the oily plant sap to insects of the family Aphididae (Zhang et al., 2006; Burgio et al., 2007), there are no results about existence of Cry protein in whiteflies bodies or honeydew. However in this study, existence of Bt proteins in the honeydew of *B. tabaci* has been proved and because honeydew of *B. tabaci* is an important nutrient source for many insects such as natural enemies (parasitoids) and ants; it is possible that Bt proteins transfer from one level of food chain to another one. The Cry proteins present in non-target insect illustrate that we still need to conduct further investigation in order to detect the Bt protein during the food chain and also evaluate its effect on the ecosystem. Many researches validated that transgenic plants in the fields affect on non-target organisms distribution. The studies about the dynamic of *A.*

gossypii in the Bt and non-Bt plants indicated that transgenic crops can affect on population dynamic of aphids (Zhang et al., 2008; Fernandes et al., 2012).

The current study showed that the Bt cotton, compared to non-Bt cotton, had significant effect on the developmental time, fecundity and body weight of *B. tabaci*. In fact, expression of Cry proteins due to the Bt genes inserting into the cotton plants, cause changes in the secondary compounds or changes in host quality responsible for herbivore/cotton plant interactions. Delay in development of *B. tabaci* rearing on the Bt cotton, is a good characteristic which makes it a proper choice for integration with other non-Bt cotton tactics such as biological control. Although it should be considered that Bt cotton may have negative effects on natural enemies of *B. tabaci*.

The results of this study demonstrated that transgenic Bt cotton has a potential to adversely affect on the abundance of secondary pests or sucking insects and can be a proper tool for control of a wide range of arthropods other than the only Cry specific targets. However, there is a need for more investigations about the possible effects of such crops on the natural enemies (parasitoids and predators) rearing on the transgenic crop pests.

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