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Biological parameters of the non-target pest *Aphis gossypii* Glover (Hemiptera: Aphididae) on genetically modified (GM) *Bt* cotton

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In the present work, we aimed to evaluate: 1) the influence of the Cry1Ac protein expressed by the genetically modified cotton variety (*Bt*) NuOpal, on the biological parameters of a non-target pest, *Aphis gossypii*, reared under laboratory conditions; 2) the influence of plant age on aphid development. Cotton cultivars were grown following technical advice. In the laboratory, the aphids were separated into plastic containers including a cotton sheet, which was changed daily with the help of a moist cotton cloth. Observations were carried out daily, and the duration of the immature and adult stages, and offspring number, were recorded. Our results show that regardless of the differences in the duration of each instar, cultivars *Bt* and non-*Bt* and the different ages of the plant to *Bt*, no differences were seen in the total duration of these phases. It was only during the reproductive period that differences were observed among genotypes for 120 days. Regarding the number of offspring and longevity total, there were differences between the different ages of the *Bt* treatment.

Key words: Biosafety, transgenic, *Gossypium hirsutum,* genetically modified (GM) plant.

INTRODUCTION

Cotton, *Gossypium hirsutum* L. is one of the most economically important crops worldwide, susceptible to a myriad of arthropod pests, which attack all stages of plant development. With a production estimated at 1.417 t/ha of cotton lint, and 2.334 t/ha of seed cotton (Fnp Consultoria and Comércio, 2009), Brazil is the fifth largest cotton producer in the world. The country's high productivity, however, is constantly threatened by pests. In the Midwest region, for instance, insecticides are applied on average 12 to 20 times on each crop field (Tomquelski et al., 2007; Fontes et al., 2006).

One economically importantly pest of cotton is the cotton aphid *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) (Gallo et al., 2002). This piercing and sucking insect lives in colonies on the underside of leaves, sucking sap from the plants, which they pierce with their sharp style (Santos, 2001). Feeding by the cotton aphid causes wrinkled, deformed leaves and buds, slows plant development and hinders plant growth, thereby reducing productivity (Silva, 1992). Secondary pest populations have increased in *Bt* cotton as they are not targeted; for example *A. gossypii* and *Bemisia tabaci* (Gennadius) (Cui and Xia, 2000; Liu et al., 2002).

Genetically modified plants expressing toxins derived from the bacterium *Bacillus thuringiensis* Berliner, 1911(*Bt*) have been cultivated over 200 million hectares

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since 1996 (James, 2009). Transgenic cotton expressing the Cry1Ac toxin, which targets the main cotton pests the pink bollworm *Pectinophora gossypiella* Saunders, 1844, the apple caterpillar, *Heliothis virescens* Fabricius, 1781 and the leaf worm *Alabama argillacea* Hübner, 1818 (Perlak et al., 2001), has been cultivated since 1996 in Australia, Mexico, Brazil Argentina, China, Colombia, India, Indonesia, South Africa and in the United States (James, 2009).

In Brazil, the *Bt* cotton was released for commercial farming in March 2005. Some restrictions, such as incorporation of exclusion zones, refuge areas, conventional cultivars corresponding to 20% of the total crop field, in addition to the generally recommended crop management practices, were enforced. *Bt* cotton plants, except for their tolerance to target insects, are theoretically equivalent to their non-transformed parental strain (CTNBio, 2005) in all agronomic and phenotypic characteristics.

The fact with the advantages of *Bt* crops are the reduced need to spray chemical insecticides for pest control, and the fact of reduced risk of adverse impacts on non-target species, some of which are natural enemies of agricultural pests. Transgenic crops are generally considered beneficial to human health and to the environment (Shelton et al., 2002; Naranjo, 2005; Huang et al., 2005; Cattaneo et al., 2006; Herdt, 2006). During the first ten years of transgenic cotton cultivation (1996 to 2006), a reduction in insecticide application on this crop was recorded worldwide (Brookes and Barfoot, 2008).

Despite the advantages mentioned above, the proportion of plant sucking insects in *Bt* cotton fields has increased, and is higher when compared with conventional cotton fields (Wilson et al., 1992; Fitt et al., 1994; Cui and Xia, 1998; Greene et al., 1999). It is possible that the *Bt* technology has an indirect, positive effect on the growth of non-target herbivore populations, for instance *A. gossypii* (Suji et al., 2008), in part because *Bt* cotton fields are sprayed less with conventional insecticides (Liu et al., 2005).

Some studies have demonstrated that *Bt* crops expressing the Cry1A toxins to control lepidopteran pests can have a direct effect on aphids (Schuler et al., 2001; Dutton et al., 2002). Contrasting with those results, Sisterson et al. (2004) found no significant differences in mortality among aphids fed transgenic cotton, and Sujii et al. (2008) found that *Bt* cotton does not significantly affect aphid life cycle, survival, fecundity and colony formation. Research carried out by Deng et al. (2003), however, put forth convincing evidence that aphid populations on *Bt* cotton are consistently larger than those on conventional cotton.

Aphids have an important role in food chains within agroecosystems, serving as food for several parasitoid and predator species (Lawo et al., 2009). Knowing the response prey/hosts populations to *Bt* crops is very important in understanding predator/parasitoid dynamics, hence natural enemy community responses.

Although previous studies have arrived at conflicting results, none has compared the toxicity of leaves from *Bt* cotton in different phenological stages.

Based on the idea that *Bt* plants may indirectly affect the non-target pests, the objective of this paper was to compare the life cycles of *A. gossypii* individuals reared on different phonological stages of two crops, the *Bt* cotton (NuOpal) and non-*Bt* cotton (DeltaOpal), under laboratory conditions.

MATERIALS AND METHODS

Seeds of the *Bt* and non *Bt* cultivars were sown in the experimental area of the Universidade Federal da Grande Dourados and grown according to agronomic best practice (Beltrão et al., 2003).

Cotton leaves containing *A. gossypii* were collected from non-*Bt* cotton, to create insect stocks. Insects were kept in the insectary of the Entomology Laboratory of the Faculdade de Ciências Biológicas e Ambientais, in a climate-controlled chamber, BOD at 25°C, 70% RH, and photophase of 12 h. Non-*Bt* cotton leaves were used as a substrate.

In the laboratory, individuals from the stock were isolated in 250 mL plastic containers closed with lids. In order to evaluate how the plant's phenological phase (at days 30, 60, 90 and 120 after emergence) affects insect development, we used a total of 60 nymphs from the first generation o*bt*ained in the laboratory (30 nymphs for *Bt*-cotton and 30 for non-*Bt*) up to 24 h old.

We used the following method to monitor the aphid's life cycle, from birth to death. We quantified the offspring of the first generation and collected the individuals with the aid of a fine-tipped brush to use them in the experiments. In order to prevent samples from drying out, we placed a moist cotton swab inside the container along with the same age leaf for each phenological phase which were changed every 24 h. We used a completely randomized design with 10 repetitions, each consisting of three insects.

The following variables were evaluated: duration of the nymph stages, duration of each instar, average longevity, offspring number and reproductive period. All variables o*bt*ained at the different ages of the plant were compared. The results o*bt*ained with the *Bt* cotton were compared with those o*bt*ained with conventional, non-*Bt* cotton. In order to compare the mean variables o*bt*ained from the two cultivars, the data o*bt*ained were subjected to analysis of variance and, when a significant difference among the means was noticed, they were compared using the t test at 5% probability. Data o*bt*ained for each phonological phase of the plant were also subjected to analysis of variance. When significant, they were compared using the Tukey's test at 5%.

RESULTS

Nymphs of *A. gossypii* created in *Bt* cotton leaves had longer duration when compared to the non-*Bt* cotton in the third and fourth instars for 90 and 120 days for the fourth instar. Exception was observed in duration of third instar with plant leaves at 120 days after emergence (DAE), and the duration for individuals created were statistically higher in leaves of non-*Bt* cotton (Table 1).

Table 1. Duration of nymph stages (days) (± SE) of *A. gossypii* leaves from *Bt* (NuOpal) and non-*Bt* (DeltaOpal) cotton, at different phonological stages.

Stage	Cultivar	Days after emergency					CV (%)
		30	60	90	120	F	
1st instar	NuOpal	1.30 ± 0.08^{a}	1.23 ± 0.07^{a}	1.23 ± 0.07^{a}	1.30 ± 0.08^{a}	0.22	35.77
	DeltaOpal	1.36 ± 0.08^{a}	1.4 ± 0.09^{a}	1.36 ± 0.08^{a}	1.41 ± 0.09^{a}	0.032	35.26
	t test	0.5399^{ns}	-1.1198^{ns}	-1.3868^{ns}	-0.9034^{ns}		
2 nd instar	NuOpal	1.20 ± 0.07^{a}	1.46 ± 0.09^{a}	1.25 ± 0.08^{a}	1.35 ± 0.10^{a}	1.94	36.87
	DeltaOpal	1.25 ± 0.08^{a}	1.41 ± 0.09^{a}	1.14 ± 0.08^{a}	1.26 ± 0.08^{a}	16.615	36.68
	t test	-0.4492^{ns}	0.9015^{ns}	0.4025^{ns}	0.6325^{ns}		
3rd instar	NuOpal	1.16 ± 0.06^{a}	1.03 ± 0.03^{b}	1.14 ± 0.08^{b}	1.04 ± 0.03^{b}	4.8	30.13
	DeltaOpal	1.11 ± 0.05^{a}	1.03 ± 0.03^{a}	1.07 ± 0.04^{a}	1.09 ± 0.07^{a}	0.5907	28.13
	t test	0.5942^{ns}	0.6942*	0^{ns}	-0.4924*		
4th instar	NuOpal	1.26 ± 0.08^{a}	1.25 ± 0.09^{b}	1.03 ± 0.03^{b}	1.04 ± 0.03^{b}	3.39	33.85
	DeltaOpal	1.04 ± 0.03^{a}	1.23 ± 0.11^{a}	1.00 ± 0.0^{a}	1.00 ± 0.0a	10.776	33.79
	t test	2.3556*	1*	0.1755^{ns}	1*		
Total nymph	NuOpal	4.96 ± 0.15^{a}	4.70 ± 0.13^{a}	4.57 ± 0.12^{a}	4.66 ± 0.10^{a}	1.37	15.51
	DeltaOpal	4.84 ± 0.16^{a}	4.88 ± 0.15^{a}	4.62 ± 0.10^{a}	4.76 ± 0.14^{a}	0.5113	16.39
	t test	0.4906^{ns}	-0.3009 ^{ns}	0.8275^{ns}	0.4619^{ns}		

Means followed by the same letter in each line do not differ according to the Tukey's test (α= 0.05). ns, t-test non-significant (α= 0.05) for both cultivars; * means differ among one another according to the t-test (α= 0.05).

The analysis of the effects of two cultivars analyzed separately by age of the plant showed differences only for nymphs reared on leaves of *Bt* cotton for the third instar; individuals from leaves of 30 days lasted the longest stage in comparison to others. For the fourth instar *Bt* treatment, there was a lesser duration of this phase, when compared with other ages.

When the duration of the reproductive phase of aphids reared on different phenological stages of the cotton plant were compared among one another, no significant differences were found (Table 2), with one exception: the reproductive phase of aphids reared on *Bt* and non-*Bt* leaves 120 DAE. showed significant differences. Also, when the duration of the reproductive phase of aphids reared on *Bt* and non-*Bt* cotton cultivars were compared, no significant differences were observed between the two treatments at the different ages of the plant. (Table 2)

With respect to offspring number, no significant differences were found when cotton cultivars were compared, because the average number of offspring was similar in both, at all phenological stages of the plant (Table 2).

However, analysis of variance revealed significant differences in the average number of aphid descendants when the parental generation was reared on different phenological stages of *Bt* plants: offspring production decreased with plant age (Table 2). The average number of aphid offspring resulting from parents reared on conventional cotton leaves of different ages was not significant.

DISCUSSION

Aphids spent on average one day in each of the four instars. Instar duration did not differ significantly among treatments, except for differences found for fourth instar larvae reared on *Bt* plants 90 days old, and for third instar larvae reared on plants 120 DAE.

Similar data for the duration of each instar were generated by Person et al. (2004), who used four varieties of conventional and Parajulee (2007) cotton to rear separated individuals directly in the plant, under temperature similar to the one used by us. Analyzing three generations, Liu et al. (2005) found that the *Bt* cotton did not affect the duration of the pre-reproductive period of *A. gossypii*. Divergent results were observed by Zhang et al. (2008), who found a shorter pre-reproductive phase for aphids reared on conventional cotton 5.9 days old, and on 6.3 days old GM cotton sold in China. Using

Table 2. Longevity (days) offspring number (± SE) of *A. gossypii* leaves from *Bt* (NuOpal) and non-*Bt* (DeltaOpal) cotton, at different phonological stages.

Stage	Cultivar	Days after emergency				F	CV (%)
		30	60	90	120		
Reproductive period	NuOpal	13.08 ± 0.60^{a}	11.81 ± 0.93^{a}	10.42 ± 0.76^{a}	10.27 ± 0.69^{a}	0.08	38.17
	DeltaOpal	12.8 ± 0.69^{a}	10.69 ± 0.09^{a}	10.16 ± 0.62^{a}	9.85 ± 0.88^{a}	22.406	41.44
	t test	0.2611^{ns}	0.7793^{ns}	0.2444^{ns}	0.3133*		
Offspring number	NuOpal	45.95 ± 2.50^{a}	42.77 ± 3.6^{a}	33.77 ± 3.16^{a}	29.39 ± 2.1^{b}	3.84	45.93
	DeltaOpal	39.84 ± 2.61^{a}	35.73 ± 6.93^{a}	33.45 ± 2.79^{a}	32.28 ± 3.18^{a}	15.484	47.69
	t test	1.5165^{ns}	1.29^{ns}	0.0716^{ns}	-0.6545^{ns}		
Total longevity	NuOpal	18.66 ± 1.46^{a}	15.16 ± 1.19^{a}	14.16 ± 0.98^{a}	13.03 ± 1.32^{b}	4.24	46.54
	DeltaOpal	15.4 ± 1.31^{a}	13.83 ± 1.24^{a}	12.33 ± 1.09^{a}	16 ± 1.01^{a}	15.623	46.31
	t test	1.6608^{ns}	0.773^{ns}	1.2436^{ns}	-1.5883^{ns}		

Temperature was 25 ± 1 °C, RH was 70 ± 10% and photo phase was 12 h. Means followed by the same letter in each line do not differ according to the Tukey's test (α= 0.05). ^{ns} t-test non-significant (α= 0.05) for both cultivars. *Means differ among one another according to the t-test (α= 0.05).

three conventional cotton cultivars and their transgenic isolines, Lawo et al. (2009) found no differences in the pre-reproductive phase of experimental cotton aphids.

Host plant characteristics, for instance appropriate nutritional contents and plant hormonal stimuli which instigate the insect to start feeding can influence the success of the development and reproduction of phytophagous insects (Fernandes et al., 2001). Suji et al. (2008) observed that the *Bt* cotton did not influence the choice of cultivar for colony formation, and the number of winged individuals produced in *Bt* and non-*Bt* plants.

In our study, the *Bt* cotton did not affect the average duration of the reproductive period of *A. gossypii*. This result is very similar to that o*bt*ained by Suji et al. (2008) in which the reproductive period (of cotton aphids) lasted 16.35 and 16.18 days on *Bt* and conventional cotton, respectively. Michelotto and Busoli (2003) found that aphid reproductive period lasted 15.5 days for specimens fed conventional cotton. In Liu et al. (2005), the reproductive phase of aphids in the first generation varied among three types of cotton. According to the authors, however, when feeding on *Bt* cotton, aphids do not ingest the insecticide protein.

Our results show that, regardless of the type of cotton, the average offspring number was similar among the different phenological ages of the host plant. Our data is not consistent with the results of Suji et al. (2008). Even though they did not observe significant differences in the average offspring number among cultivars (*Bt* and non-*Bt*), a higher total mean offspring per female, for *Bt* (47.26) when compared with non *Bt* (46.98), did result from their experiment. According to Michelotto and Busoli (2003), the number of offspring produced per female was 84 nymphs in the DeltaOpal cultivar. Pessoa et al. (2004) found a total production of aphids between 54 and 69 individuals. Similar values were observed by Funichello et al. (2012), who reported an average offspring of 63 for NuOpal and 59 for Deltaopal. However, Lawo et al. (2009), in India, observed that the total fertility of the cotton aphid is closer to that of the present study, between 22 and 38 nymphs, not differing significantly between treatments. In China, Zhang et al. (2008) compared the biological parameters of *A. gossypii* reared on *Bt* and non-*Bt* cotton, and o*bt*ained an average of 30 and 29 nymphs produced per female in the respective treatments.

We do not know why the number of offspring is inversely proportional to the age of the plant; it might be the result of less favorable conditions for the establishment of *A. gossypii* populations as plants age. According to Degrande (1998), the attacks by the cotton aphids begin 15 days after plant emergence, and are especially intense between 40 and 70 days, when populations are densest. Gonzaga et al. (1991) concluded that the vertical distribution of pest populations on the cotton plant vary with plant age. Aphids are detected up to 90 days after plant emergence, but are not found afterwards.

The results of this study suggest that the longevity of the cotton aphid was not affected by the type of cultivar. However, the average longevity of aphids in this study was lower than that reported by Suji et al. (2008). In their data, aphids lasted on average 20.47 days on *Bt* cotton and 20.98 days on non-*Bt* crop, respectively. Fuchinello et al. (2009) also did not observe any kind of effect of transgenic cotton on the biological parameters of aphids,

finding a higher average life span for those on *Bt* crops (21.83) when compared with individuals reared on non-*Bt* cotton (24.40 days). Michelotto and Busoli (2003) observed that *A. gossypii* reared on the cultivar DeltaOpal lived, on average, 24.33 days. Lawo et al. (2009) evaluated the performance of aphids on plants in the three varieties of transgenic *Bt* cotton (Mech 12, Mech 162, Mech 184) most commonly grown in India, and their respective isolines. In their results, aphid longevity was 24, 21 and 24 days for the three cultivars, and 22.8, 21.5 and 25.3 days for *Bt* crops, respectively. As longevity is one of the biological characteristics that can be more easily affected by toxins ingested by the insect, we hypothesize that the pest is not much affected by the Cry1Ac toxin.

Literature data suggests that the two most common methods of rearing aphids, that is directly on the plant, or detaching the plant leaves and keeping them with the insect inside a laboratory chamber do not interfere with the ecology of these insects. However, after analyzing the extensive literature available (Michelotto and Busoli, 2003; Suji et al., 2008; Fuchinello et al., 2009; Lawo et al., 2009), we suggest that the total longevity of the cotton aphid was affected by the methodology adopted in this work.

It is possible that the amino acid contents on different parts of a plant species vary according to the phenological stage of plant cycle, and plant variety (Fernandes et al., 2001). When considering the different phenological phases of the *Bt* cotton at 30 and 120 days, there were significant differences in the average longevity of *A. gossypii*.

Even though toxins produced by *Bt* plants affect insect targets and some non-target insects (Dogan et al., 1996; Hilbeck et al., 1998; Dutton et al., 2002), these substances are often not detectable. Some authors consider that the *Bt* endotoxins expressed in plants are not found in the phloem of the plant, or have been detected in the aphid (Raps et al., 2001; Dutton et al., 2002). By contrast, Zhang et al. (2008) reported having found the toxin in aphids reared on *Bt* cotton. The authors, however, did not investigate the possible effects of the toxin on this pest.

The results of this study show that *Bt* cotton did not exert any influence on the biological parameters of *A. gossypii*, because those parameters were equivalent with those o*bt*ained from organisms reared on conventional cotton. It is important to highlight, however, the need to study the possible effects of *Bt* cotton o tri-trophic interactions, evaluating the possible accumulation of this toxin in natural enemies.

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