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Full Length Research Paper

Tritrofic interactions between Bt cotton plants, the aphid *Aphis gossypii* Glover, 1827 (Hemiptera: Aphididae), and the predator, *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae)

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This research examined the effects of the Cry1Ac toxin on the chrysopid predator *Chrysoperla externa*, fed *Ashbya gossypii*-aphids reared on cotton (Bt) NuOPAL and non-Bt DeltaOPAL (Bollgard™) for two generations. Individual eggs from the predator were placed in glass containers, and hatched. Each treatment consisted of 20 replicates, each containing one insect. Larvae in treatment 1 were fed *A. gossypii* individuals reared on a diet of NuOPAL (Bt) cotton leaves. Larvae in treatment 2 were given aphids of the same species previously fed leaves of conventional DeltaOPAL cotton. The average duration of larval instars, pre-pupal and pupal phases from both treatments (Bt and non-Bt) and between generations were evaluated using the t test at 5% probability. The duration of the larval instars in treatment 2 was longer than in treatment 1, but these differences were significant only for the second and third instars. In the second generation, a significant difference in the duration of the first instar and the larval phase were observed. However, when comparing the data on the duration of the second instar, the first generation predators in treatment 1 had a shorter lifespan. The average viability was 96.29% for non-Bt treatment against 91.07% for individuals in the Bt treatment. The results of this study suggests that the biology and development of *C. externa* larvae fed aphids reared on Bt cotton leaves were not affected by the Bt-toxin, possibly because these aphids do not accumulate the Bt-toxin.

Key words: Chrysopidae, biological control, genetically modified plant.

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) is found in all continents, and is particularly abundant in the tropics, where it attacks many crops. Both wingless and winged forms live under leaves and young shoots, sucking plant sap. Cotton aphids have a rapid reproductive capacity and in tropical regions reproduce exclusively by thelytokous parthenogenesis (Gallo et al., 2002). The cotton aphid is considered an important pest of the initial phenological phases of the cotton (Arantes et al., 1998).

Among the insects that stand out as predators of agricultural pests, lacewings of the family Chrysopidae are of particular interest, because they have high predatory capacity and ecological plasticity. For this reason, chrysopids are found in a variety of ecosystems

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(Lira and Batista, 2006), and feed on a variety of prey items such as aphids, whiteflies, eggs and small caterpillars, thrips and mites. Adults of *Chrysoperla* Steinmann (1964) feed on plant products (Carvalho and Souza, 2000). Several studies have demonstrated the predatory potential of *Chrysoperla* larvae using different prey under laboratory conditions (Figueira et al., 2000; Maia et al., 2004; Dos Santos et al., 2005; Bortoli et al., 2006). Among the species occurring in Brazil, *Chrysoperla externa* (Hagen, 1861) seems to hold considerable potential for biological control (Albuquerque et al., 1994).

The use of genetically modified insect-resistant plants expressing Cry protein crystals is an important tool in integrated pest management (IPM). However, high doses of Cry proteins in plant tissues may represent a potential hazard to non-target herbivorous insects, and to their natural enemies (Wu and Guo, 2003). In the current scenario, practically all countries in the world that have significant agricultural activity grow at least one transgenic crop (James, 2006).

The Bt cotton allows farmers to reduce the cost of insect control (Perlak et al., 2001). In Brazil, Bollgard™ 531, cotton (also known as Bollgard™, produced by Monsanto Ltd containing the Cry1Ac gene), was approved for commercial release by CTNBio No. 0513/2005 - Bt Cotton, following CTNBio Normative Instruction No. 10 of 19/02/98 and the biosecurity law No. 11,105 (Praça et al., 2007).

Many herbivores consume Cry proteins and survive. However, predators that consume such herbivores may be adversely affected due to tritrophic interactions, because these substances tend to concentrate in the bodies of organisms up the food chain (Sisterson et al., 2004). The sequestration of Bt proteins by non-target herbivores with low susceptibility indicates that these proteins can be transferred between trophic levels and may interfere with the food web (Torres et al., 2006). The possible deleterious effects on non-target herbivores and their predators may have important implications for the use of genetically modified organisms in biological control (Lundgren and Wiedenmann, 2005). Based on the IPM model, tritrophic interactions involving transgenic plants in sustainable agricultural production systems must be carefully analyzed.

Studies on the tritrophic interactions between the predator *C. carnea* (Stephens) and the prey *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) found that predators consuming caterpillars contaminated with the Bt toxin had a reduced longevity when compared with predators eating caterpillars reared on a diet free from the toxin (Hilbeck et al., 1999; Dutton et al., 2002).

Furthermore, in those studies, predators maintained on an artificial diet containing the Bt-toxin also lived longer than their counterparts preying on items reared on Bt-crop (Hilbeck et al., 1998b). Similar results using *C. carnea* were obtained when O*strinia nubialis* (Hübner) (Lepidoptera: Crambidae) caterpillars were used as prey: those reared on Bt-corn (containing the Cry1Ab gene) were associated with a shorter predator lifespan when compared with their counterparts reared on conventional corn (Hilbeck et al., 1998a).

Obrist et al. (2006) argued that the prey is also affected by the Bt protein, and that the differences in predator longevity found in those studies may be explained, at least in part, by the relatively lower nutritional value of prey items reared on Bt-corn.

Recognizing the need to know more about the interactions between plant resistance and natural enemies, we set out to investigate the tri-trophic interactions involving the predator *C. externa* and their prey *A. gossypii*. We compared the lifespan of two generations of predators given a diet of aphids reared on cotton (Bt) NuOPAL and non-Bt DeltaOPAL.

MATERIALS AND METHODS

The experimental area was tailored to the chemical and biological needs of cotton plants. There was no artificial irrigation, as being common for cotton crop in the Mid-West region of Brazil. The seeds used were Bt cotton (NuOPAL) and non-Bt cotton (DeltaOPAL) cultivars, each representing one treatment. Sowing took place on 01/31/2010, following the recommended practice for this kind of cultivar (Embrapa, 1997).

In order to avoid larval cannibalism, individual eggs of *C. externa* were placed—in glass containers (2.5 × 8.5 cm) sealed with polyethylene film (Dos Santos et al., 2005). Our experimental design consisted of two treatments. Each treatment had 20 replicates, each replicate consisting of one predatory larva. For establishing the replication number, we based this research on the study of Pessoa et al. (2004) and Costa et al. (2002). In Treatment 1, predators were offered *A. gossypii* cotton aphids reared on NuOPAL (Bt) cotton leaves. In Treatment 2, aphids reared on conventional cotton leaves DeltaOPAL were offered as prey. Predator larvae in their 1st, 2nd, and 3rd instars were fed a total of 14, 30 and 90 prey items per day, respectively (Ribeiro, 1988; Pessoa et al. (2004). The treatments were conducted in the Laboratory of Entomology in B.O.D. chambers at 25°C, RH 70% and photophase of 12 h (Figueira et al., 2000); conditions considered most suitable for *C. externa* immatures.

The emerging adults were separated in pairs according to their respective treatments into cylindrical PVC breeding cages (100 mm in diameter). Each cage was coated with A4 type white bond paper to serve as a substrate for oviposition, and supported by trays. The upper region of each cage was sealed with voile-type fabric to allow for good aeration. Distilled water was provided to the adults using humidified cotton packed in 10 ml bottles.

A mushy diet containing yeast and honey (1:1) was applied daily on the top of the cage. Second-generation eggs, resulting from those couples, were individualized, and when larvae hatched, they were subjected to the same treatment as their parents.

The data among mean duration of larval instars, pre-pupae and pupae in the two treatments (Bt and non-Bt), and between generations, were compared using the t-test (5% probability).

RESULTS

With one exception, mentioned subsequently as this

Table 1. Duration of immature stages (days ± standard error) of the first generation of *Chrysoperla externa* fed *Aphis gossypii* reared on Bt crops (NuOPAL) and non-Bt (DeltaOPAL).

Cultivar	Duration (days)					
	1st instar	2nd instar	3rd instar	Larval phase	Pre-pupe	Pupe
NuOpal	3.3 ± 0.10	3.05 ± 0.08	3.36 ± 0.01	9.73 ± 0.22	2.38 ± 0.13	6.562 ± 0.19
DeltaOpal	3.15 ± 0.10	3.3 ± 0.14	3.8 ± 0.30	9.95 ± 0.29	2.41 ± 0.21	7.31 ± 0.46
t -test	0.9885^{ns}	-1.4595*	-1.2018*	0.141^{ns}	-0.086^{ns}	-1.3209*

Temperature was 25 ± 1°C, RH was 70 ± 10% and photophase was 12 h. ns, Non-significant t-test (α= 0.05); *means differ according to the t-test (α = 0.05).

Table 2. Duration of immature stages (days ± standard error) of the second generation of *C. externa* fed *A. gossypii* reared on Bt crops (NuOPAL) and non-Bt (DeltaOPAL).

Cultivar	Duration (days)					
	1st instar	2nd instar	3rd instar	Larval phase	Pre-pupe	Pupe
Nuopal	3.25 ± 0.14	3.36 ± 0.13	3.55 ± 0.278	9.83 ± 0.46	2.16 ± 0.11	7.17 ± 0.34^{a}
DeltaOpal	3.52 ± 0.15	3.16 ± 0.23	3.61 ± 0.33	10.33 ± 0.31	2.7 ± 0.15	6.81 ± 0.57^{a}
t -test	-0.3664*	0.7168^{ns}	-0.1208^{ns}	-0.8475*	-2.6407*	0.4972^{ns}

Temperature was 25 ± 1°C, RH was 70 ± 10% and photophase was 12 h. ns indicates non-significant t-test (α= 0,05); *means differ according to the t-test (α = 0.05).

study proceeds, the development of each immature instar of *C. externa* was longer in Treatment 2 (prey reared on DeltaOPAL cotton), than in Treatment 1 (prey reared on NuOPAL (Bt) cotton). In the first instar, this pattern was not observed. The differences between the two treatments were statistically significant (α = 0.05) for the 2nd and 3rd instars, and for the pupal phase (Table 1).

Overall, the differences in the duration of the various instars did not influence the average duration of the entire larval period of *C. externa*. This result can be explained because the longer duration of the first instar recorded for predator larvae in the Bt treatment may have being compensated for the shorter span of the remaining instars. It may be noted that the duration of the pre-pupal period did not differ significantly among treatments.

In the second generation of *C. externa*, the first instar, the larval phase and the pre-pupal phase lasted significantly longer in individuals fed *A. gossypii* maintained on conventional cotton (Treatment 2) than in those given aphids reared on Bt cotton (Treatment 1) (Table 2).

The other parameters of *C. externa* were not influenced by the type of diet given to their prey. No negative effects from the Bt cotton were observed on lacewing on larval development time (days), indicating that the transgenic plant diet of the aphids does not influence the predator.

When the two generations of lacewings fed prey reared on Bt cotton aphids were compared, no significant differences in the duration of the larval instars of *C. externa* were observed, even though the larval and pupal periods of individuals in the first generation were significantly shorter than of individuals in the second generation. That is, under the conditions tested, it was not possible to observe the influence of the transgenic cultivar on the biological parameters of *C. externa* larvae. The results suggest that the biological aspects of the larval stage of predators fed the two different diets did not differ between generations (Table 3).

The results on the average viability of each biological stage of *C. externa* show differences mainly in the pupal stage of the test individuals favoring the conventional farming. However, average viability of pupae was higher in the first generation than in the second; the viability for the conventional crop was 96.29%, against 91.07% for the Bt treatment. The viability of the first generation in treatment 1, 85.7% (Bt) was well below that found for the conventional treatment, 96.29% (Table 4).

The average viability of individuals in the two generations was 100% viability for the first instar in treatment 1 (Bt) against 98.3% in Treatment 2 (non-Bt). The latter treatment resulted in 100% viability of individuals in the 3rd instar, against 96.6% for the Bt treatment. The viability of the second instar was similar in both treatments (Table 4).

DISCUSSION

Risk assessment in insect-plant interactions involving transgenic plants and natural enemies should include investigations on natural enemies' exposure and their susceptibility to the plant toxins. Species at higher trophic levels got contaminated after eating food containing the active insecticidal protein (Obrist et al., 2006). The two treatments in this study, conducted across two generations, did not reveal an influence of the Bt crop on the

Table 3. Comparison between the larval periods of two generations of *C. externa* fed *A. gossypii* (days ± standard error) reared on Bt cotton (NuOPAL) and non-Bt (DeltaOPAL).

Cultivar	Duration (days)					
	1st instar	2nd instar	3rd instar	Larval phase	Pre-pupe	Pupe
NuOpal 1st generation	3.3 ± 0.10	3.05 ± 0.08	3.36 ± 0.01	9.73 ± 0.22	2.38 ± 0.13	6.56 ± 0.19
NuOpal 2nd generation	3.25 ± 0.14	3.36 ± 0.13	3.55 ± 0.27	9.83 ± 0.46	2.16 ± 0.11	7.17± 0.34
t -test	0.282^{ns}	-1.975^{ns}	-0.5399^{ns}	-0.259*	-0.086^{ns}	-14.042*
CV (%)	17.04	16.08	30.15	24.85	18.79	16.18
DeltaOpal 1st generation	3.15 ± 0.10	3.3 ± 0.14	3.8 ± 0.30	9.95 ± 0.29	2.41 ± 0.21	7.31 ± 0.46
DeltaOpal 2nd generation	3.52 ± 0.15	3.16 ± 0.23	3.61 ± 0.33	10.33 ± 0.31	2.7 ± 0.15	6.81 ± 0.57
t-test	-1.9602^{ns}	0.4765^{ns}	0.4069^{ns}	-1.3582^{ns}	-1.0426^{ns}	0.6051^{ns}
CV (%)	18.63	26.32	38.06	32.18	32.75	13.41

Temperature was 25 ± 1°C, RH was 70 ± 10% and photophase was 12 h. ns, Non-significant t-test (α = 0.05); *, means differ according to the t-test (α = 0.05).

Table 4. Viability of the instars, larval period, pre-pupae and pupae of *C. externa* fed *A. gossypii* reared on Bt (NuOPAL) and non-Bt (DeltaOPAL) cotton, for two generations (± standard error).

Cultivar	Viability (%)					
	1st instar	2nd instar	3rd instar	Larval phase	Pre-pupe	Pupe
NuOpal 1st generation	100	100	96.66	96.66	96.55	85.7
NuOpal 2nd generation	100	96.66	96.55	96.33	100	96.42
Mean	100 ± 00	98.3 ± 1.66	96.6 ± 0.05	95 ± 1.66	98.2 ± 1.7	91.07 ± 5.3
DeltaOpal 1st generation	100	100	100	100	90	96.29
DeltaOpal 2nd generation	96.66	96.55	100	93.33	96.42	96.29
Mean	98.3 ± 1.6	98.2 ± 1.7	100 ± 00	96.6 ± 3.3	93.2 ± 3.2	96.29 ± 00

Temperature was 25 ± 1°C, RH was 70 ± 10% and photophase was 12 h.

larval parameters of the predator *C. externa*. Predator development was normal, with three instars, pre-pupa and pupa, as observed by previous studies using the same predator, but different prey items (Ribeiro, 1988; Figueira et al., 2000; Fonseca et al., 2000). The biological characteristics of *C. externa* larvae and their predatory potential are not affected by the Bt crop.

The results of this work regarding average duration of each instar in the two treatments were generally consistent with those reported by Costa et al. (2002) and Pessoa et al. (2004), testing conventional cultivars, except for the third instar, which had a longer duration in the two generations.

When the two generations were compared, there were differences in the duration of the larval phase of individuals fed aphids reared on Bt crops. These differences did not occur in the treatment using conventional cotton. Given this result, an influence of the Cry1Ac toxin at this stage cannot be ruled out. As *C. externa* is a predator only in the larval stage, any interference with the duration of this phase may influence its success in biological control.

The results on the duration of the larval period, pre-pupa and pupa resemble those of Dos Santos et al. (2005), who observed, for those stages, a duration of 10.1, 3.1 and 7.0 days, respectively.

The pupal stage was not significantly longer for individuals of the first generation in treatment 2 (conventional cotton), and second generation individuals from this treatment had a shorter pupal stage with respect to the Bt treatment. This difference, however, was not significant. By contrast, the duration of the pupal stage differed significantly between the generations of *C. externa* in treatment 1, which might indicate that the toxin can accumulate and influence these individuals.

The viability obtained in our data, greater than 90% for the three instars regardless of cotton cultivar, is consistent with the data from Dos Santos et al. (2005), but our values for the larval stage were higher than those reported by Pessoa et al. (2004), who found that larvae of *C. externa* aphids fed the conventional cop had 67.3% viability. This study shows that there were no differences in the viability of lacewings, regardless of the cultivar used to nourish the prey. The lowest viability found for individuals of the first generation in the pupal stage subjected to the NuOpal treatment may not be due to the

influence of the Bt toxin, because the viability of individuals in the second generation was close to that found for the conventional treatment.

Data from this study suggest that the biology and development of *C. externa* larvae fed aphids reared on Bt cotton leaves did not suffer any deleterious effect. It is possible that this group of predators is not affected by the Bt toxin or that the toxin is not present in toxic levels in the body of the prey. This observation confirms those of Dutton et al. (2002), who studied the effect of the toxin Cry1Ab on the chrysopid *C. carnea* fed *Rhopalosiphum padi* (L.) and *Tetranychus urticae*, finding no influence on the biological parameters of the predator.

Hilbeck and Meier (2001), studying tritrophic interactions between the predator *C. carnea* and two non-target herbivore species, *S. littoralis* (Lepidoptera: Noctuidae) and *R. padi* (Hemiptera: Aphidae), found that third instar predators had a significant preference for *S. littoralis* individuals feeding on non-Bt corn, but did not display any preferences with regards to *R. padi*. Zhang et al. (2008) found no significant differences in the biology of the predator *Orius sauteri* (Poppius) fed *A. gossypii* which is reared on conventional *versus* Bt cotton. They detected, however, that predators in their 4th instar consumed less aphids on average, when the latter came from non-Bt cotton.

Zwahlen et al. (2000) gave the predator *O. majusculus* (Reuters) specimens of *Anaphothrips obscurus* (Muller, 1776) reared on non-Bt and Bt corn. They found no significant differences in the biological parameters of predators fed prey items from either crop.

Unlike laboratory tests, agro-ecosystems offer predators a greater range of prey items. They can choose what to eat, thereby changing their exposure to Bt toxins. It is possible that the biological cycle of *C. externa* is not affected by this type of trophic interaction during intense aphid infestation, because the predators will search for this alternative prey (Men et al., 2003; Whitehouse et al., 2005).

Finally, more studies are needed in order to determine the cumulative effect of transgenic crop toxins on *C. externa* over many generations, because differences in some larval parameters can significantly influence the number of generations during the phenological cycle of the crop.

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