

Full Length Research Paper

Effect of transgenic and non-transgenic cotton cultivars on the development and survival of *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae)

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The aim of this work is to evaluate the main biological aspects of *P. includens* fed on four cotton cultivar leaves and to study the effect of the transgenic cultivar NuOPAL (Bollgard I[®]) which express the protein Cry1Ac on the survival and development of the insect, *Pseudoplusia includens*. Two assays were carried out using the completely randomized blocks design. The former was represented by the cultivars DeltaOPAL, NuOPAL, FMX 993 and FMX 910 used for feeding the insect larvae, and the latter assay, only the cultivar NuOPAL and its conventional isoline DeltaOPAL were used for larvae rearing, with a higher larvae population. The leaves were obtained from cotton plants cultivated in the field and placed in Petri dishes where neonate larvae were individualized and fed daily with the respective cultivars. The following biological parameters were evaluated: duration period, weight and viability of larvae and pupae, duration and viability of larva-pupa-adult period, longevity of adults and sex ratio. Bt cultivar (Bollgard I) suppressed the half of larval population, extends the larval stage of the survivors, reduced the larvae and pupae weights and provides significantly higher duration of larva-adult period.

Key words: Soybean looper, *Gossypium hirsutum*, Bt cotton, genetically modified organisms (GMO), Cry1Ac.

INTRODUCTION

Cotton crop is an important commodity in Brazilian agricultural scenario by its volume and production value, bearing great social importance due to the number of employments generated directly and indirectly. However, cotton crop may be affected by several insect pest species, which can significantly compromise its production (Jacome et al., 2003). As an alternative for the rotation with soybean crop, Brazilian Midwest region growers found in cotton and maize a great business opportunity. However, the soybean looper *Pseudoplusia*

includens (Walker) (Lepidoptera: Noctuidae), most frequently on soybean crop until the last decade, has caused considerable economical losses for cotton in the last years (Busoli et al., 2011).

Pseudoplusia includens is a specie with wide geographic distribution and may occur from the northernmost part of the United States to the northernmost part of South America (Alford and Hammond Jr., 1982). This insect may feed on several plant species, including some which are of agricultural

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importance, such as soybean, cotton, common bean, tobacco, sunflower and some vegetables (Bueno et al., 2007). In Brazil, *P. includens* became a major soybean pest in the last years (Bueno et al., 2007), and its incidence on cotton is increasingly higher every year.

Their larvae feed on leaves located in the lower third of the plants, and in the first instars they select tender leaves, feeding on those with lower fiber amount, and became less demanding as they develop. After the third instar, *P. includens* larvae consume extended leaf areas, remaining however the main ribs, which confers a tracery aspect to injured leaves (Herzog, 1980). Among the main advances towards controlling cotton pests, the availability of genetically modified cultivars with genes from the bacteria *Bacillus thuringiensis* Berliner stands out. Through this technology, the genetically modified cotton was obtained, called Bollgard I[®], whose plants produce the α -endotoxin from *B. thuringiensis* var. *kurstaki*, highly pathogenic to most lepidopterous larvae (Ramiro and Faria, 2006).

Li et al. (2005) while evaluating the effect of Bt cotton that expresses the proteins Cry1Ac and Cry2Ab on the survival and development of *Trichoplusia ni* (Hübner), observed that the larvae fed on the transgenic cotton showed lower survival, negatively influencing their development. However, some Lepidoptera larvae species had low susceptibility to protein Cry1Ac, as *Spodoptera frugiperda* (J. E. Smith) and *S. exigua* (Hübner), which had relatively suppressive control by this protein due to the prolongation of larval stage and lower fecundity (Stewart et al., 2001; Adamczyk and Gore, 2004).

Another important factor, according to Maia (2010), is that the major environmental risk associated with transgenic crops is the evolution of the resistance in target pests. Thus, the strategy known as 'refugee area' is recommended, which refers to the use of a hybrid or cultivar that express the Bt toxin in high concentration in all plant tissues combined to the adoption of refuges, that is, a part of the entire area occupied by non-transgenic host plants.

Therefore, the aim of this work was to evaluate the main biological aspects of *P. includens* and to study the effect of the transgenic cultivar NuOPAL (Bollgard I[®]) on the behavior, survival and development of the insect, enabling the discussion about the influence of such results on the refugee theory for the management of resistant populations to the technology.

MATERIALS AND METHODS

Cultivation process

The assays were carried out in the experimental area of Fazenda de Ensino, Pesquisa e Produção (FEPP) and in the Laboratório de Manejo Integrado de Pragas (LAMIP) of the Departamento de Fitossanidade (FCAV/UNESP), Jaboticabal, SP, Brazil. The research area is located between the latitude 21°14'15" S and longitude 48°17'09" W and 615 m altitude. The experiments were

carried out during November 2010 and October 2011, in an area of 5400 m² where the commercial cotton cultivars DeltaOPAL, NuOPAL (Bollgard I[®]), FM 910 and FM 993 were sown.

The experimental area soil was prepared through one plowing and two disking using the fertilizer N-P-K (8-20-20) (400 kg ha⁻¹). Sowing was performed manually in November 19, 2010, with a density of 14 non-treated seeds per linear meter, spaced at 0.9 m between rows. Plant emergence occurred in November 27, 2010, and 20 days after the emergence, thinning was done, keeping an average density of 10 plants per linear meter. During the experiment, there was not any application of insecticides, fungicides or herbicides, and manual weeding was performed throughout plant development.

Pseudoplusia includens rearing and maintenance

Pseudoplusia includens rearing was initiated from eggs of population kept in artificial medium and maintained under climatized conditions (25 ± 1°C, 70 ± 10% relative humidity and 12 h of photophase) in LAMIP. After hatching, larvae were transferred into plastic containers of 7.0 cm diameter and 5.0 cm in height, with 100 ml volume capacity, where approximately 20 ml of the artificial medium was put in liquid in each container which solidified afterwards at the bottom. The artificial medium was prepared based on white beans, brewer yeast, wheat germ and casein, according to Greene et al. (1976) methodology.

Three larvae were transferred per container, where they remained until pupation. Next, pupae were put into oviposition cages constituted by 20.0 cm diameter and 20.0 cm height PVC tubes, covered in the top with *voile* tissue and lined inside with bond paper in order to allow egg laying by the adults. At the bottom of the cage, a 14.0 cm diameter plastic plate lined with bond paper with the same diameter of the cage was inserted. For adult feeding, honey solution at 10% impregnated in a cotton wad was put inside the cage, and changed daily to avoid contamination by microorganisms. During the oviposition, the eggs laid in the *voile* and bond paper were removed daily and transferred into a 7.0 cm diameter and 5.0 cm height with 100 ml volume capacity plastic containers with a 27.0 cm³ of the solidified artificial medium, and closed until the larvae eclosion. Afterwards, they were transferred into the rearing containers, previously described, through a watered wet paint brush.

Biological aspects of *P. includens* on four cotton cultivars

Neonate larvae from the maintenance rearing were individualized into Petri dishes of 9.0 cm in diameter, where they were fed daily with young leaves of the cultivars DeltaOPAL, NuOPAL, FM 910 and FM 993 until they reached pupal stage. For each treatment (cultivars), 30 replications were used in the completely randomized blocks design. Laboratory conditions were 25 ± 1°C of temperature, 70 ± 10% of relative humidity and 12 h of photophase. The main biological aspects of *P. includens* assessed were as follows: duration and viability of larval stage, weight of 12-day-old larvae, duration and viability of pupal stage, weight of 24 hours-old pupae, longevity of adults without feeding and duration and viability of the cycle larva-pupa-adult.

RESULTS

The average duration of *P. includens* larval period was 22.23 days, significantly higher in the transgenic cultivar NuOPAL, with lower larval viability of 56%, whereas the larval stage duration in the non-transgenic cultivars were

Table 1. Duration (days) and viability (%) of larval stage, weight (g) of 12-day-old larvae, duration (days) and viability (%) of pupal stage and weight (g) of 24 hours-old pupae of *Pseudoplusia includens* fed on cotton cultivars leaves. Jaboticabal, SP, Brazil, 2011.

Cultivar	Larval stage			Pupal stage		
	Duration (days)	Viability (%)	Weight (g)	Duration (days)	Viability (%)	Weight (g)
DeltaOPAL	17.68±0.28 ^b	83 ^{ab}	0.603±0.27 ^{bc}	7.20±0.59 ^b	80 ^a	2.228±0.06 ^a
NuOPAL	22.23±0.45 ^a	56 ^b	0.482±0.26 ^c	8.68±0.46 ^a	76 ^a	1.921±0.07 ^b
FMX 993	16.81±0.28 ^b	90 ^a	1.599±0.14 ^a	8.48±0.11 ^a	95 ^a	2.399±0.05 ^a
FMX 910	17.39±0.30 ^b	80 ^{ab}	0.906±0.08 ^b	8.39±0.17 ^a	85 ^a	2.472±0.05 ^a
F	37.51 ^{**}	3.84 ^{**}	21.26 ^{**}	20.92 ^{**}	1.15 ^{ns}	8.81 [*]
CV (%)	9.69	52.26	59.49	8.69	42.33	15.46

Mean followed by the same letter in the column did not differ significantly by Tukey test, at 5% probability. ^{ns}Not significant; ^{**}significant at 1% probability.

Table 2. Duration (days) and viability (%) of larva-adult period, longevity (days) and sex ratio of *Pseudoplusia includens* fed on cotton cultivars. Jaboticabal, SP, Brazil, 2011.

Cultivar	Larva-adult period		Longevity (days)	Sex ratio
	Duration (days)	Viability (%)		
DeltaOPAL	27.91±0.19 ^{ab}	63 ^a	2.35 ^b	0.38
NuOPAL	30.05±0.44 ^a	46 ^a	2.69 ^b	0.41
FMX 993	25.39±0.51 ^c	73 ^a	3.27 ^a	0.56
FMX 910	26.71±0.58 ^{bc}	60 ^a	3.27 ^a	0.45
F	11.37 ^{**}	1.53 ^{ns}	10.29 ^{**}	-
CV (%)	9.46	80.00	21.55	-

Mean followed by the same letter in the column did not differ significantly by Tukey test, at 5% probability.

^{ns}Not significant; ^{**}significant at 1% probability.

significantly lower, with at least 5 days shorter (Table 1). The larval stage viability was up to 80% for the non-Bt cultivars, where the isoline DeltaOPAL obtained 83% of survival, and the cultivars FM 910 and FM 993 respectively, had 80 and 90% of larvae survival (Table 1). Regarding the weight of 12-day-old larvae, the cultivar FM 993 provided the highest mean value (1.599 g) and the cultivars NuOPAL (0.482 g) and DeltaOPAL (0.603 g), had the lowest weights of larvae (Table 1).

The transgenic cultivar NuOPAL showed higher mean duration of survived larvae compared to its conventional isoline DeltaOPAL. However, there were not significant differences on the parameters larval stage viability and weight of 12-day-old larvae. Regarding the pupal stage, mean duration of 7.20 days was observed, being shorter in the cultivar DeltaOPAL, whereas the pupal viability did not show significant differences among the cultivars, with values ranging from 76% (NuOPAL) to 95% (FM 993). These results show that the survived larvae turned normally into pupae, and the Bt cultivar NuOPAL had larval mean duration significantly longer and lower larvae survival.

It was also verified that the 24 hour-old pupae mean weight (1.921 g) was lower on the cultivar NuOPAL (Table 1). From these results, the toxic protein Cry1Ac affected the biological parameters of *P. includens* in the

larval stage, however, causing low larval viability, differently from *Alabama argillacea* which had 100% mortality in the study conducted by Costa et al. (2011). The duration of larva-pupa-adult period showed significant differences on the biological parameters among the cultivars. Larvae fed on the cultivar NuOPAL had mean duration significantly longer (30.05 days) in relation to the larvae fed on the cultivars FM 993 and FM 910 (25.29 and 26.71 days, respectively). The overall mean viability of larva-adult period did not show significant differences among the cultivars, although the values ranged from 46% (NuOPAL) to 73% (FM 993) (Table 2).

The average longevity of *P. includens* adults showed differences among the cultivars, and DeltaOPAL and NuOPAL had significantly shorter periods, with mean durations of 2.35 and 2.69 days, respectively in relation to the cultivars FM 993 and FM 910 (3.27 days for both). The sex ration was not significantly affected by the cultivars, with values varying between 0.38 (DeltaOPAL) and 0.56 (FM 993) (Table 2).

DISCUSSION

The effect of cotton cultivar on the behavior and biology

of insect pests is important for the development of efficient strategies within an Integrated Pest Management (IPM) program, preventing the occurrence of resistant populations to this technology. Accordingly, Santos and Torres (2010) evaluated the efficacy of the genetically modified cotton (Bt) (Deltapine Acala 90) on the control of *S. frugiperda* and *A. argillacea*, and they concluded that the protein Cry1Ac is efficient to control *A. argillacea*, but it does not suppress *S. frugiperda* population; similar to the results found for *P. includens* in the present study, although it is indicated as a target pest of the cultivar. The average duration of larva-adult period observed in this work ranged from 25.39 days for FM 993 to 30.05 on NuOPAL. The results of longer duration of *P. includens* larval stage were due to toxic protein Cry1Ac in the leaves of this cultivar. In similar bioassays conducted with the same cultivars, Costa et al. (2011) reported 100% mortality of first and second instars in *A. argillacea* larvae, indicating the presence of this protein in the leaves of these cultivars plants. The toxin Cry1Ac is related to high mortality of defoliating larvae, such as *A. argillacea*, however, not causing suppression of *P. includens* and *S. frugiperda* larvae on cotton plants in Brazil. When the larvae of these species survive, they show alterations on the biological parameters, e.g., on the duration of larval stage, extending it and providing lower weight because of the low food intake.

As observed in this study, the toxic protein Cry1Ac did not affect the parameters of pupal stage duration and pupae viability as the mode of action is directly on the larval stage by the feeding and toxic effect of the protein on the epithelium of larvae midgut, causing septicemia and death of the susceptible larvae or alterations on the feeding behavior of those less susceptible to the protein crystal. However, the protein affected the weight of pupae, probably by the negative influence on the feeding behavior of the insects during the larval stage, reflecting in less developed pupae. The genetically modified cotton cultivars with only the production of the protein Cry1Ac are not considered efficient against some lepidopterous species, however, they may act providing the prolongation of larval stage and lower larvae weight (Stewart et al., 2001).

Studies of Morales et al. (1995) indicated significant differences on the susceptibility of *Anticarsia gemmatalis* Hübner and *P. includens* to *B. thuringiensis* strains, whereby *A. gemmatalis* was from 1.5 to 5.6-fold more susceptible to the bioinsecticides composed by *B. thuringiensis* HD-1 and BD-1 strains than *P. includens*. For *P. includens* larvae that were fed on leaves from Bt NuOPAL and non-Bt DeltaOpal cultivars, it was observed that the transgenic cultivar showed significantly longer mean duration of the survived larvae than its conventional isolate, as well as lower weight of 12-day-old larvae and larval viability than the non-Bt cultivar DeltaOPAL.

These results are clear evidence that the transgenic

cultivar affects *P. includens* larvae development, not causing, however, mortality of part of their population. Time use of this technology is highly dependent on insect resistance management programs to the toxic protein from *B. thuringiensis*, such as growing of refuge areas adjacent to the cultivated area. Thus, this technology can be used in IPM programs as a supplementary tactic to control *P. includens* as a moderate resistant plant. However, under high population densities, as it occurs in cotton fields in Brazilian Cerrado regions, this technology might not be efficient in the control of this pest, which infests the plants simultaneously with other defoliating larvae, demanding insecticide applications and inflating the production cost.

The transgenic cultivar NuOPAL suppress part of *P. includens* population on cotton, acting negatively on some biological parameters of the insect, extending the larval stage of the survived larvae, reducing the weight of larvae, resulting in lower weight pupae and longer larva-adult period, in addition to its negative influence on the refuge theory in managing the resistant larvae, once the insects from non-Bt cultivars showed significantly shorter cycles than those which survived the Bt technology, not occurring synchronism of adults populations from transgenic and conventional cultivars.

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