Effect of varieties of GM cotton on *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) larvae

Efecto de variedades de algodón genéticamente modificadas sobre larvas de *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae)

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Rec.: 06.06.2013 Accep.: 14.01.2014

Abstract

Spodoptera frugiperda, is a polyphagous pest with economic importance in tropical and subtropical countries. In Colombia, *S. frugiperda* is a secondary pest in cotton. This cultivar has gained importance due to the adoption of genetically modified crops (GM). The objective of this study was to determine the sub-lethal effects of GM cotton varieties on *S. frugiperda* larvae. In order to do so, NuOPAL RR (Cry1Ac), DP141 B2RF (Cry1Ac+Cry2Ab) and DeltaOPAL RR (No-Bt) varieties were tested under laboratory controlled conditions (27 °C, 65 ±10% RH and 12 h photoperiod). The parameters to be evaluated were: (a) weight of larvae, (b) weight of feces, (c) weight of provided food and, (d) weight of non-consumed food. Digestibility and consumption indexes were calculated: Relative Consumption Rate (RCR), Relative Growth Rate (RGR), Efficiency of the Conversion of Ingested Food (ECI) and the Approximate Digestibility (AD). The nutritional indexes show antifeedant activity of DP141 B2RF, (Cry1Ac+Cry2Ab), affecting in a negative way the growth rate and the conversion of larvae food. The weight gained by larvae fed with DP141 B2RF (Cry1Ac+Cry2Ab) was 58.6% less than the control. These results suggest an unfavorable effect of DP141 B2RF (Cry1Ac+Cry2Ab) in the larval development of *S. frugiperda*. The antibiotic effects were confirmed observing minor development and a negative effect in the survival of larvae fed on GM cotton.

Key words: Antifeedant activity. Bt cultivars, *Gossypium hirsutum*, nutritional indexes, *Spodoptera frugiperda*.

Resumen

Spodoptera frugiperda es una plaga polífaga de importancia económica en países tropicales y subtropicales. En Colombia ataca el algodonero (*Gossypium hirsutum* L.), cultivo que ha tomado importancia gracias a la adopción de variedades genéticamente modificadas (GM). En el estudio se determinaron los efectos subletales de algunas de estas variedades sobre larvas de este insecto. Para el efecto, en condiciones de laboratorio (27 °C, 65 ± 10% HR y 12 h fotoperiodo) se evaluaron las variedades NuOPAL RR (proteína Cry1Ac), DP141 B2RF (proteínas Cry1Ac+Cry2Ab) y DeltaOPAL RR (no Bt). Los parámetros evaluados fueron: (a) peso de larvas, (b)peso de heces, (c) peso del alimento ofrecido y (d) peso del alimento no consumido. Como índices de consumo y digestibilidad se calcularon la tasa relativa de consumo (TRCo), la tasa relativa de crecimiento (TRCr), la eficiencia de conversión del alimento ingerido (ECI) y la digestibilidad aproximada (DA). Los índices mostraron actividad antialimentaria de la variedad DP141 B2RF (Cry1Ac+Cry2Ab) que afecta de forma negativa la tasa de crecimiento y la conversión del alimento por parte de las larvas. El peso de las larvas alimentadas en DP141 B2RF (Cry1Ac+Cry2Ab) fue 58.6% menor que en la variedad no Bt. Los resultados muestran un efecto adverso de la variedad DP141 B2RF (Cry1Ac+Cry2Ab) en el desarrollo larval de *S. frugiperda*. Se confirman los efectos antibióticos de las variedades GM evaluadas que inciden en un menor desarrollo y en la supervivencia de las larvas.

Palabras clave: Actividad antialimentaria, cultivares Bt, *Gossypium hirsutum*, índices nutricionales, *Spodoptera frugiperda*.

Introduction

The armyworm (*Spodoptera frugiperda*) is one of the most important polyphagous pest from an economic point of view in South America. Its economic importance lies in the impact it has on various crops in Colombia and in other countries (Jaramillo *et al.*, 1989, Velez, 1997). The damage caused to crops such as corn and cotton, at all stages of the crop stands out (Velez, 1997, Zenner *et al.*, 2005, Santos *et al.*, 2009).

In cotton the attack can be presented during all stages of the crop, consuming young leaf tissue on top of the plant or on the ground till stem lignification, then it will feed on foliage; at the time of formation of the buttons the larvae attack these structures from the base to the apex (García, 1975, Murillo, 1991). In Colombia there is still no precise information on the losses caused by insect attack; however in the case of the techified maize crop, it is estimated that between 5% and 10% of the production costs are related to the chemical control of this pest (Zenner *et al.*, 2007, 2009).

GM plants to which the Cry1Ac gene from *Bacillus thuringiensis* var. Kurstaki has been inserted have insecticidal properties against larvae of some species of Lepidoptera of the families Noctuidae and Gelechiidae such as *Heliothis virescens* Fabricius and *Helicoverpa zea* Boddie, these GM cotton varieties are known by the name Bollgard (Ellsworth *et al.*, 1995a, Ellsworth *et al.*, 1995b, Waquil *et al.*, 2002, Tabashnik *et al.*, 2003). The international literature indicates that *S. frugiperda* is no pest of concern in temperate countries, for which the varieties of GM cotton were developed, which is why in these regions basic studies recommended for insect were not performed (EPA 1998).

With the planting of modified varieties an adjustment in the community of insects associated with cotton is expected, in response to the elimination or reduction of populations of species susceptible to the protein (Cry1Ac) and the reduction of applications of insecticides. However, one risk associated with this technology is the emergence of secondary species that can colonize free niches due to mortality of species sensitive to these modified materials (Altieri, 2000). Among the potentially benefited species, the *Spodoptera* complex (Bacheler and Mott, 2003) is cited. Zenner *et al.* (2008) found that susceptibility to the toxin of larvae of *S. frugiperda* and *S. sunia* collected in Tolima was negligible and concluded that the modified cotton currently grown in Colombia provide a satisfactory control of bollworms but not of the *Spodoptera* complex, which corroborates the results found by Adamczyk *et al.*, 1997 and Berrocal *et al.*, 2005.

The development of efficient strategies for the control of insects such as *S. frugiperda* requires knowledge of their biology and their relationship with their host. Quantitative analysis of the consumption and use of plants is a commonly used tool in studies of plant-insect interactions (Scriber and Slansky, 1981). Given the findings related to the lower susceptibility of *S. frugiperda* to certain GM cotton materials, the objective of this study was to determine the effects of two varieties planted in Colombia (Bollgard and Bollgard II®) on development, survival and efficient feed utilization by *S. frugiperda*.

Materials and methods

Location and cotton varieties

The tests were carried out at the Centro Internacional de Agricultura Tropical (CIAT), 3° 31' N and 76° 22' W, at 965 m.a.s.l. and annual rainfall of 1000 mm. Cotton varieties used were NuOPAL RR expressing the gene for Bt protein Cry1Ac (Bollgard ®), DP141 B2RF expressing the genes for proteins Cry1Ac and Cry2Ab (Bollgard II®) and DeltaOPAL RR as control (non-Bt variety). The varieties were seeded in a mesh house, in plastic containers with a capacity of approximately 10 kg containing a mixture of soil and sand in the ratio 1: 1. Plantings were made about 45 days before the start of the insect feeding trials.

S. frugiperda culture

In commercial plots of corn planted at CIAT, insect larvae were collected and fed with castor (*Ricinus communis* L.). With the first generation (F1) a laboratory insect colony was established and fed with non-Bt cotton leaves (variety DeltaOPAL RR) in order to prepare them for the consumption of this plant, in which it is a secondary pest. With larvae of the second generation (F2) feeding trials were set with each of cotton varieties. The insects were kept under controlled laboratory conditions (27 °C, 65 ± 10% RH and 12 h photoperiod). In all cases, the adults had available a solution of honey and water in the ratio 1: 3.

Growth and development indices

These indices were: (1) survival rate of immatures, (2) weight of pupae 24 h after observing the change from pre-pupa to pupa, (3) weight gain of larvae, (4) Dry weight of feces produced by the larvae, and (5) days till adult emergence or time between birth of larvae and birth of adults.

Feeding trials to measure antibiosis

For this test, 50 newly emerged larvae were individualized in a 2-ounce container for each of the three varieties of cotton. The change of food was done every two days and with the same frequency weights larvae, feces, offered food and uneaten food were measured. Feces and the unconsumed feed fraction were dried for 96 h to 65 °C. The weight was taken until the last larva in each variety of cotton finished feeding; subsequently pupal weights were taken, differentiating between males and females. For each larva a control cup (no larva) was included, with a known leaf fraction to determine the weight loss by evaporation, this leaf was also changed every 48 h and placed in an oven to determine its dry weight. The trials consisted of four replications in time until 195 larvae were evaluated for each variety. Before starting each repetition the entire process of collection and pre-adaptation of the larvae was done as described above, in order to work only with second generation larvae. With the data obtained the following indices were calculated, with the methodology proposed by Waldbauer (1968) and modified by Scriber and Slansky (1981):

1. Relative growth rate, $RGR = (FW-IW) / (AW \times T)$ where FW is the final larval weight (g), IW initial larval weight (g), AW is the average weight of larvae during the test (g) and T is the duration of the feed period (days). This parameter represents the gain (g) of biomass per day of the insect in relation to its weight (g).
2. Relative consumption rate, $RCR = I / (AW \times T)$, where I is the food ingested (g).
3. Efficiency of conversion of ingested food, $ECI = (FW - IW) / I \times 100$. It represents a measure of the efficiency of the digestive and metabolic processes
4. Approximate digestibility, $AD = (I - F)/(I \times 100)$, where F is the feces weight (g). It represents the percentage of ingested food that is actually absorbed by the insect. The test was performed in a complete randomized design and the analysis by means of ANOVA and least

significant difference (LSD) of Fisher (P <0.05).

Results and discussion

Growth and development indices

The survival of the larvae was different (P < 0.05) when fed with GM varieties. S. frugiperda larvae fed with leaves from the DP141 B2RF variety presented a survival of 3.4%, lower than the value obtained when fed with the variety NuOPAL RR (35.0%) and with the non-Bt variety DeltaOPAL RR (53.3%) (Table 1).

These results show that the specific action of the endotoxins synthesized from the genes Cry2Ab the Cry1Ac was lethal to the larvae, which coincides with the results found for larvae of *S. frugiperda* collected from different regions of the US and Brazil and other species of Lepidoptera as *H. zea* and *S. exigua* Hübner (Adamczyk Jr. *et al.*, 2001, 2008, Gore *et al.*, 2001). De Sousa Ramalho *et al.* (2011) found that survival of larvae fed with Bt cotton (Bollgard) was 22.6% lower than that observed for larvae fed with non-Bt cotton. Other studies in laboratory and field conditions show that Bt cotton (Bollgard II®) is effective as an insecticide for control of larvae of *H. virescens, H. zea* and other Lepidoptera species including *S. frugiperda* and favor the decline of insect populations densities in cotton(Gore *et al.*, 2001, Marchosky *et al.*, 2001, Shelton *et al.*, 2002, Wu *et al.*, 2003, Adamczyk Jr. *et al.,* 2008, Greenberg y Adamczyk, 2010, Greenberg *et al.,* 2010, 2011).

The length of the days to adult emergence of *S. frugiperda* was affected by the variety of cotton from which the larvae fed; time that was longer in larvae fed the variety DP141 B2RF (43.8 days). This effect favors crop development due to the reduction in the number of insect generations per crop cycle. In varieties DeltaOPAL RR and NuOPAL RR no differences were observed (P > 0.05) in the duration of days to adult emergence (36.5 and 33.8 days, respectively) (Table 1). These results agree with those obtained by De Sousa Ramalho *et al.* (2011) who observed a difference of 2.8 days in the larval stage between Bt and non-Bt cotton.

A significant effect was observed (P < 0.05) of the variety DP141 B2RF on the weight of *S. frugiperda* pupae. Pupae fed with this material showed a weight 1.34 times lower than the weight of those fed with the non-Bt variety DeltaOPAL RR (Table 1), which is consistent with the results found by De Sousa Ramalho *et al.* (2011), with larvae fed with Bt cotton (Bollgard).

Table 1. Effect of GM cotton varieties (NuOPAL RR Bollgard® and DP141 B2RF Bollgard II®) and non-Bt variety (DeltaOPAL RR) on developmental characteristics of *S.frugiperda.*

Characteristic	**Cotton varieties**		
	DeltaOPAL RR (No Bt)	**NuOPAL RR (Cry1Ac)**	**DP141 B2RF (Cry1Ac + CRy2Ab2)**
Immature survival (%)	53.3±4.67 a*	35.0±5.89 b	3.4±1.10 c
Days till adult emergence (days)	36.5±0.89 b	33.8±0.78 b	43.8±1.08 a
Pupal weight (mg)	183.1±0.00 a	157.2±0.00 b	136.6±0.00 c
Dry weight of larvae feces (mg)	126.2±0.01 a	113.7±0.01 a	52.4±0.01 b
Live weight gain of larvae (mg)	222.4±0.02 a	169.5±0.01 b	86.0±0.01 c

*Values followed by the same letter within each row, are not significantly different (P < 0.05), Fisher DMS).

The larvae that fed with the leaves of the variety DP141 B2RF produced feces with dry weights of 52.4, a lower value than the one obtained from larvae fed with the non-Bt variety DeltaOPAL RR (126.7 mg) and NuOPAL RR (113.7 mg) (Table 1). These values are twice lower than those found by De Sousa Ramalho *et al.* (2011) with *S. frugiperda* larvae fed with a Bollgard cotton variety. This behavior was similar for the weight gain by immature individuals (P < 0.05). The larvae fed with the variety DP141 B2RF had a weight 58.6% less than the weight of the larvae fed with the non-Bt variety (Table 1).

The studies of Armstrong *et al.* (2011) show that *S. frugiperda* larvae feed on varieties with Bollgard II® technology, but toxins in these varieties consequently affect their growth and development. Fernandes *et al.* (2003) also found that when feeding the larvae of *S. frugiperda* with MON810 maize (Cry1Ab), the weight was reduced compared with larvae fed with conventional corn, due to the negative effect of the Cry1Ab protein on the larvae.

Feeding trials

The amount of food eaten or relative consumption rate (RCR) did not differ (P > 0.05) between varieties of cotton, indicating that the larvae consume the food equally, but this does not mean that assimilation occurs; this is confirmed by the growth rate, especially from the larvae in GM varieties DP141 B2RF and NuOPAL RR that presented a negative effect on the feeding of larvae of *S. frugiperda*, being the highest inhibitory effect for the first (0.11 g/g/day) (Table 2).

The efficiency of conversion of ingested food (ECI) was lower for the variety DP141 B2RF (45.9%) compared with the non-Bt material DeltaOPAL RR (66.3) (P < 0.05), suggesting that the components of the plant material have a negative impact on the larvae and its ability to take nutrients; in short, despite the larvae feed on GM varieties, they do not metabolize the food consumed, therefore there is no efficient conversion into biomass.

The reduction in the percentage of the ECI in variety B2RF DP141, relative to non-Bt variety DeltaOPAL RR is possibly due to the concentration and use of energy for regenerating the midgut epithelium damaged by endotoxins (Liithy and Wolfersberger, 2000), instead of larval growth. The results of this study are similar to those found by Chen *et al.* (2005) for *Helicoverpa armigera* (Hubner), where the larvae fed with Bt cotton drastically reduced the conversion of ingested food.

With variety DP141 B2RF, there was a reduction in the conversion of ingested food and daily biomass gain in *S. frugiperda* larvae, causing a drop in production of feces and weight gain; while with the variety NuOPAL RR, a negative effect was observed on the biomass gain per day and in the

Table 2. Effect of GM cotton varieties (NuOPAL RR Bollgard® y DP141 B2RF Bollgard II®) and non-Bt variety (DeltaOPAL RR) on growth, consumption and use of the feed of *S. frugiperda* larvae.

Variety	RCR (g/g/day)	RGR (g/g/day)	CIF (%)	AD (%)
DeltaOPAL RR (No Bt)	1.358±0.09 a*	0.184±0.01 a	66.372±3.43 a	47.179±1.99 b
NuOPAL RR (Bollgard®)	1.141±0.10 a	0.152±0.01 b	90.806±4.73 a	38.335±2.76 c
DP141 B2RF (Bollgard® II)	1.328±0.12 a	0.116±0.01 c	45.923±4.14 b	70.784±3.08 a

RCR: Relative Consumption Rate, RGR: Relative Growth Rate, CIF: Conversion of Ingested Food, AD: Aproximate digestibility

*Values followed by the same letter within each column are not significantly different (P < 0.05), Fisher DMS).

approximate digestibility, results that match those found by De Sousa Ramalho *et al.* (2011) in *S. frugiperda* larvae fed with Bollgard® cotton. Although Bollgard® varieties do not show a strong effect on larval survival, this study shows that the presence of the protein Cry1Ac cause physiological changes.

The approximate digestibility (AD) was different between varieties (P <0.05); variety DP141 B2RF was 1.5 times more digestible than non-Bt variety DeltaOPAL RR. In studies with other lepidopterans, it was found that the largest AD value is associated with greater retention of food in the insect gut, allowing an increase in digestion and absorption of nutrients (Koul and Isman, 1991, Rossetti *et al.*, 2008). For the variety NuOPAL RR this parameter was 1.2 times lower compared with the non-Bt variety, matching results of Schmidt *et al.* (1997), in larvae of Spodoptera Eridania (Cramer), who associate this decrease in AD with the damage caused by the extracts on epithelial cells and smooth muscle of the larval midgut. Other authors such as Boucias and Pendland (1998) consider that the low values of AD are because the food is not being retained in the gut of the larvae. The quick passage of food through the digestive tract of the insect reduces the interaction of the proteolytic enzymes on the food bolus, which causes a drop of the active toxins in the gut lumen (Dinglasan *et al.*, 2009). In works of Prütz and Dettner (2004), the low consumption of leaves of Bt varieties is attributed to the partial paralysis of the midgut, caused by the presence of endotoxins in the digestive tract of the larvae.

Larvae fed with the variety DP141 B2RF always showed a slower growth and a longer duration of this stage of development, therefore these used energy for maintenance and not for conversion into biomass, which brought as a consequnce its death in the attempt of turning to pupa and completing their life cycle. The lethal effect of variety DP141 B2RF on larvae of *S. frugiperda* was due to the low weight of the pupae, low feed conversion efficiency and the low percentages of adult emergence when compared to the results obtained with the non Bt variety DeltaOPAL RR. Therefore, the lower growth of the larvae of *S. frugiperda* is the result of a deficiency in the assimilation and conversion of nutrients into biomass body, when fed with Bollgard® cotton varieties.

Conclusions

- The characteristics of low consumption and poor nutritional value, and the toxic effect caused by modified cotton varieties on larvae and adults of *S. frugiperda*, provide relevant information on the side effects of Bt technology to the test conditions.
- Under the test conditions, the combination of the proteins Cry1Ac and Cry2Ab in the variety B2RF DP141 was effective in controlling the false bollworm (*Spodoptera frugiperda*).
- The variety B2RF DP141 had the greatest impact on the larval development of *S. frugiperda*, confirming the antibiotic effects of GM crops on the development and survival of these larvae.

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