

Feeding of fall armyworm, *Spodoptera frugiperda*, on Bt transgenic cotton and its isoline

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Abstract

Studies on insect food intake and utilization are important for determining the degree of insect/plant association and host species' resistance, and also for helping design pest management programs by providing estimates of potential economic losses, techniques for mass breeding of insects, and identifying physiological differences between species. We studied the feeding and development of fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), on transgenic (Bt) and non-transgenic (non-Bt) cotton. The larvae of *S. frugiperda* fed on Bt cotton had a longer development period (23.0 days) than those fed on non-Bt cotton (20.2 days). Survivorship of *S. frugiperda* larvae fed on Bt cotton (74.1%) was lower than that of larvae fed on non-Bt cotton (96.7%). Pupal weight of larvae fed on Bt cotton (0.042 g) was lower than that of larvae fed on non-Bt cotton (0.061 g). The cotton cultivar significantly affected food intake, feces production, metabolization, and food assimilation by *S. frugiperda* larvae. However, it did not affect their weight gain. Intake of Bt-cotton leaf (0.53 g dry weight) per *S. frugiperda* larva was lower than the intake of non-Bt-cotton leaf (0.61 g dry weight). Larvae fed on Bt-cotton leaves produced less feces (0.25 g dry weight) than those fed on non-Bt-cotton leaves (0.37 g dry weight). Weight gain per *S. frugiperda* larva fed on Bt-cotton leaves (0.058 g dry weight) was similar to the weight gain for larvae fed on non-Bt-cotton leaves (0.056 g dry weight). The cotton cultivar significantly affected the relative growth, consumption, and metabolic rates, as well as other nutritional indices: the figures were lower for larvae fed on Bt-cotton leaves than for larvae fed on non-transgenic cotton leaves.

Introduction

Fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), occurs mainly in tropical and subtropical regions (Rojas et al., 2004) and is also found in cotton- and maize producing regions of the USA and Canada (Meagher & Nagoshi, 2004). It is one of the most common pests of cotton and maize crops in the Brazilian Neotropical Savanna. The larvae of *S. frugiperda* damage cotton crops from plant emergence to maturity (Santos, 2001), attacking stems, leaves, buds, and bolls (Luttrell &

Mink, 1999). In the early stages, they prefer scraping bud bracts. When mature, they can be found inside the flowers or at the base of bolls, which they scrape and perforate. In Mato Grosso, maize is planted side by side with cotton, and the two crops share common pests. *Spodoptera frugiperda* often migrates between maize and cotton crops (Santos, 2001). The cotton yield lost to this pest is between 30% (controlled populations) and 90% (uncontrolled populations; Santos, 2001).

In general, pesticides are recommended for controlling *S. frugiperda* (Viana & Costa, 1998), although this approach can cause serious environmental problems (Parra et al., 2002), and also lead to the emergence of resistant individuals (Diez-Rodríguez & Omoto, 2001). There is an extensive documentation on the use of biopesticides as an

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alternative to chemical control. The bacterium *Bacillus thuringiensis* Berliner has drawn most attention from researchers as a lepidopteran pest control agent, as it is beneficial both in terms of cost and from an environmental perspective. Indirect benefits of conserving the agroecosystem include increasing natural enemy populations, improving secondary pest control, and decreasing pollution levels (Edge et al., 2001).

Plants genetically modified with genes of *B. thuringiensis* var. *kurstaki* (Bt) express protein crystals (Cry) during sporulation, which are lethal when ingested by lepidopteran and dipteran larvae (Schnepf et al., 1998; Polanczyk, 2004). Plants containing Bt toxin are being produced for controlling *Mocis latipes* (Guenée), *Plutella xylostella* (L.), *Heliothis* spp., and *Spodoptera* spp. (Vega, 1999). Researches suggest that genetically modified plants containing Bt toxin have great potential for controlling *S. frugiperda* (Adamczyk et al., 2001; Polanczyk, 2004). According to Adamczyk & Gore (2004), cotton cultivars enhanced with Cry1F and Cry1F combined with Cry1Ac are highly toxic to *S. frugiperda*. However, cotton cultivars containing only Cry1Ac are inefficient for controlling *S. frugiperda* (Adamczyk et al., 1998) and also increase the tolerance of individuals that feed on the cultivar (Adamczyk & Sumerford, 2001).

Depending on the host species, plants can affect insect life history in many ways (Panizzi & Parra, 1991). The quality and quantity of food consumed by the insect can affect its entire biology, impacting its development, and food intake, as well as its survivorship, reproduction, and longevity (House, 1962; Scriber & Slansky, 1981; Bavaresco et al., 2004; Meagher et al., 2004).

An insect's nutritional requirements change during the course of its development, and these changes are typically reflected in altered food intake and feeding behavior (Barton Browne, 1995). Quantitative nutritional studies can be used to identify inhibitory substances and stimulants of the host plant and to assess its nutritional quality. These studies evaluate indices of food utilized, digested, assimilated, metabolized, and converted into body matter to verify larval preferences for plant species (Scriber & Slansky, 1981). Studies on insect food utilization and intake are important for determining the degree of insect/plant association, the host species' resistance, and also for helping design pest management programs by providing estimates of potential economic losses (Costa et al., 2006), techniques for mass rearing of insects (Souza et al., 2001; Busato et al., 2004; Costa et al., 2006), and identifying physiological differences between species (Busato et al., 2002). Thus far, there is no information on the quantitative nutrition of *S. frugiperda* on Bt-cotton plants. Therefore, the aim of this study was to assess food intake, digestion,

and utilization of Bt cotton expressing Cry1Ac by *S. frugiperda* larvae and also its growth and development indices.

Materials and methods

Cotton cultivar and growing conditions

Cotton plants expressing the gene for the Bt protein Cry1Ac [cultivar DP 404 BG (Bollgard; Monsanto, Santo Louis, MO, USA)] and its non-transgenic isolate (DP 4049) were used in this study. All cultivars were planted in plastic pots (20 cm diameter, 30 cm high) and placed in a greenhouse, at 28 ± 2 °C, $70 \pm 10\%$ r.h. and L12:D12 photoperiod. One hundred and fifty plants of each cotton cultivar were distributed randomly inside the greenhouse and rerandomized once per week to minimize position effects.

Insect feeding

Egg masses of *S. frugiperda* were obtained from the Emb-rapa Algodão Biological Control Unit, and the larvae hatched in an incubator chamber, at 25 °C, $70 \pm 5\%$ r.h., and L12:D12 photoperiod (40 000 lux active radiation produced by 40-W fluorescent lamps).

When the plants reached the stage of 8–10 leaves per plant (20–30 days old), the leaves were collected and given as food to the recently hatched larvae under the temperature, humidity, and photophase conditions described above. The experiment was a randomized block design with two treatments: (1) larvae fed with DP 404 BG cultivar leaves and (2) larvae fed with DP 4049 non-transgenic isolate leaves, with 12 replications. Each experimental unit consisted of 10 larvae, with the larvae kept individually in plastic containers (80 mm diameter). Eight leaves were randomly collected from each cotton cultivar, with four replications per cultivar. These leaves were oven-dried at 80 °C for 72 h and used to calculate the proportion of dry matter and water content immediately prior to the insect rearing trials. At the same time, fresh leaves were weighed and supplied daily to each larva. Every day, the feces and remaining parts of the leaves were collected, weighed, and dried at 80 °C for 72 h. The fresh weights of the leaves and insects were quantified every other day. Larval development was calculated as the period from hatch to pupation. Pupae were weighed 12 h after pupation was noted. Larval survivorship was calculated as the number of larvae reaching the pre-pupal stage divided by the number of first instars for each treatment.

Growth and development indices

Three indices were used to measure larval growth and development, viz., the duration of larval development, pupal weight, and survivorship rate.

Food intake and utilization indices

The gravimetric method was used to determine the nutritional indices (Waldbauer, 1968), modified by Scriber & Slansky (1981): $B = (I-F)-M$, where B = metabolized food, I = food consumed (ingested), F = undigested food + excretory products (feces), M = weight gain, and $I-F$ = assimilated food (all in g dry weight). When divided by $B_x \times T$, where B_x = mean weight during the time period T (in days), the above amounts were converted to relative rates for comparative purposes: RGR (relative growth rate), RCR (relative consumption rate), and RMR (relative metabolic rate = $B/B_x \times T$). Food utilization efficiency was determined and expressed as a percentage: AD (approximate digestibility = $(I-F)/I$; assimilation efficiency), ECD (efficiency of conversion of digested food = $B/(I-F)$; net growth efficiency), ECI (efficiency of conversion of ingested food = B/I ; gross growth efficiency), and MC (metabolic cost = $100-ECD$). Thus, $RGR = RCR \times ECI$ and $ECI = AD \times ECD$. The quantity of food ingested, feces produced, and weight gains by the larvae were calculated in terms of dry weight. The weight gain by the larvae was calculated by subtracting the final dry weight of pre-pupae from the initial dry weight of the newly hatched larvae. The initial mean dry weight of larvae was estimated by weighing and killing 10 newly hatched larvae and also 10 newly hatched larvae from the group used in an experiment (Bt and non-Bt cotton). The same method was applied for calculating the amount of food eaten.

Correction for changes in leaf moisture content

The fresh weight of the leaves consumed was corrected for changes in moisture content during the period in which the food was exposed to the larvae. The weight correction method involved taking account of the loss or gain in moisture content by the leaves (Candy & Baker, 2002). The corrected weight of the food consumed was calculated as: F (correction factor) = (initial weight of control leaf) / (final weight of control leaf). The corrected weight of the food consumed was calculated as: (initial weight of leaf eaten - final weight of leaf eaten * F) / [$F - 1$] / $(\ln(F))$. For a more detailed discussion of the correction method, see Candy & Baker (2002).

Analysis of data

Analysis of variance (SAS, 2006) was used to analyze cultivar effects on population indices of *S. frugiperda* (larval development, pupal weight, and larval survivorship), consumption, and feces. Larval consumption and digestibility index data were analyzed using covariance analysis with the initial weight as a covariate of RCR, RGR, and ECI. Food intake was a covariate of ECI for correcting the effect

of variation in growth, and food assimilated for variation in consumption and growth (Raubenheimer & Simpson, 1992). Food assimilated was also used as a covariate to analyze ECD. The differences between mean values were determined using the F-test ($\alpha = 0.05$).

Results

Larval development, survivorship, and pupal weight

The larvae of *S. frugiperda* fed on Bt cotton had a longer development period (22.96 days) than those fed on non-transgenic cotton (20.16 days). Survivorship of *S. frugiperda* larvae fed on Bt cotton (74.1%) was lower than that of larvae fed on non-transgenic cotton (96.7%). Pupal weight of larvae fed on Bt cotton (0.042 g) was lower than that of larvae fed on non-transgenic cotton (0.061 g; Table 1).

Food intake, utilization, metabolism, and assimilation

Cotton cultivar significantly affected food intake, feces production, and food metabolized and assimilated by *S. frugiperda* larvae. However, it did not affect their weight gain (Figure 1). Intake of Bt-cotton leaf (0.53 g dry weight) per *S. frugiperda* larva was lower than intake of non-Bt-cotton leaf (0.61 g dry weight; Figure 1). Larvae

Table 1 Mean (\pm SD) life-history parameters and nutritional indices for fall armyworm fed on Bt and non-Bt cotton

Parameter	Cultivar		$F_{1,11}$
	Bt cotton	Non-Bt cotton	
Life history			
Larval development (days)	22.96 \pm 4.99	20.16 \pm 3.09	32.21
Larval survivorship (%)	74.12 \pm 10.13	96.67 \pm 11.89	76.99
Pupal weight (g)	0.042 \pm 0.004	0.061 \pm 0.008	71.67
Nutritional index ¹			
RGR (g g ⁻¹ per day)	0.46 \pm 0.09	0.56 \pm 0.08	112.50
RCR (g g ⁻¹ per day)	4.72 \pm 0.82	6.11 \pm 0.79	84.51
RMR (g g ⁻¹ per day)	1.42 \pm 0.56	3.03 \pm 0.71	278.22
AD (%)	40.06 \pm 5.92	58.73 \pm 6.99	124.25
ECD (%)	15.87 \pm 2.74	24.92 \pm 3.42	120.78
ECI (%)	6.39 \pm 1.98	14.63 \pm 2.98	123.73
MC (%)	75.08 \pm 8.98	84.13 \pm 9.02	120.78

$P < 0.0001$ in all cases (Student–Newman–Keuls test).

¹All weights are in g dry weight.

RGR, relative growth rate; RCR, relative consumption rate; RMR, relative metabolic rate; AD, approximate digestibility; ECD, efficiency of digested food conversion into body matter; ECI, efficiency of ingested food conversion into body matter; MC, metabolic cost.

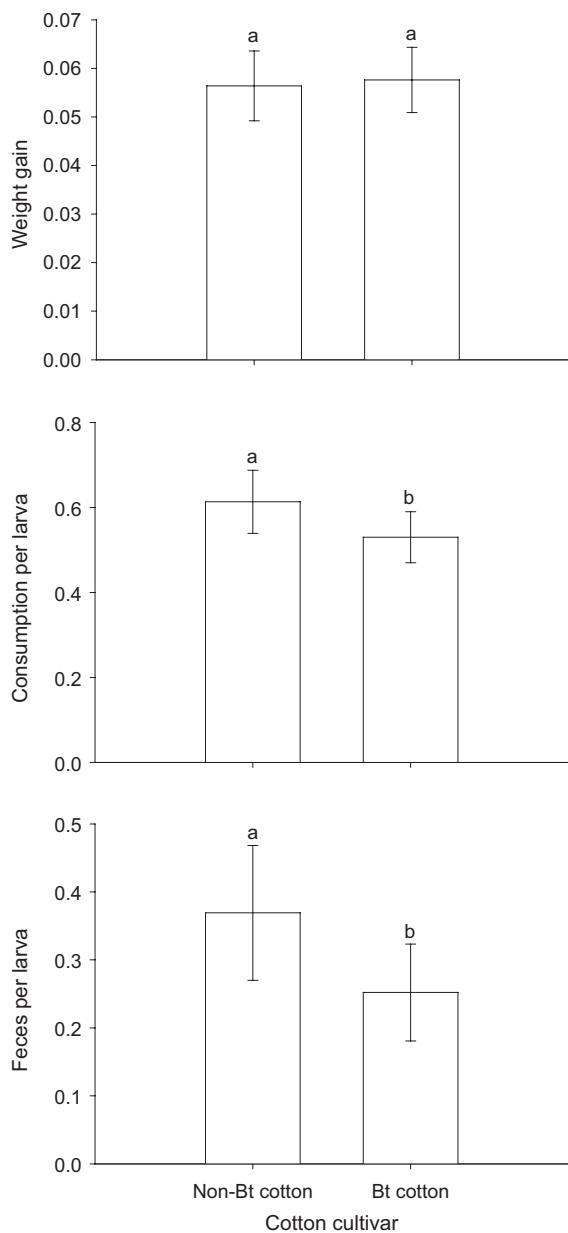


Figure 1 Mean (\pm SD) weight gain (g dry weight; $F_{1,11} = 1.54$, $P = 0.24$), consumption (g dry weight; $F_{1,11} = 4.86$, $P = 0.043$), and feces (g dry weight; $F_{1,11} = 70.88$, $P = 0.0001$) per larva of fall armyworm fed on non-Bt and Bt cotton. Different letters capping the bars within a panel indicate a significant difference between non-Bt and Bt cotton (Student–Newman–Keuls test: $P < 0.05$).

fed on Bt-cotton leaves produced less feces (0.25 g dry weight) than those fed on non-Bt-cotton leaves (0.37 g dry weight; Figure 1). Weight gain per *S. frugiperda* larva fed on Bt-cotton leaves (0.058 g dry weight) was similar to the

weight gain for larvae fed on non-Bt-cotton leaves (0.056 g dry weight; Figure 1).

Metabolized leaf of Bt cotton (0.19 g dry weight) per *S. frugiperda* larva was lower than that of larvae fed on non-Bt-cotton leaves (0.31 g dry weight; Figure 2). *Spo-doptera frugiperda* larvae fed on Bt cotton assimilated less food (0.25 g dry weight) than larvae fed on non-Bt-cotton leaves (0.36 g dry weight; Figure 2).

Nutritional indices

The cotton cultivar significantly affected all nutritional indices for larval food consumption and utilization. The figures for relative growth rate (RGR), relative consumption rate (RCR), relative metabolic rate (RMR), approximate digestibility (AD), metabolic cost (MC), efficiency of ingested food conversion (ECI), and efficiency of digested food conversion (ECD) were lower for larvae fed on

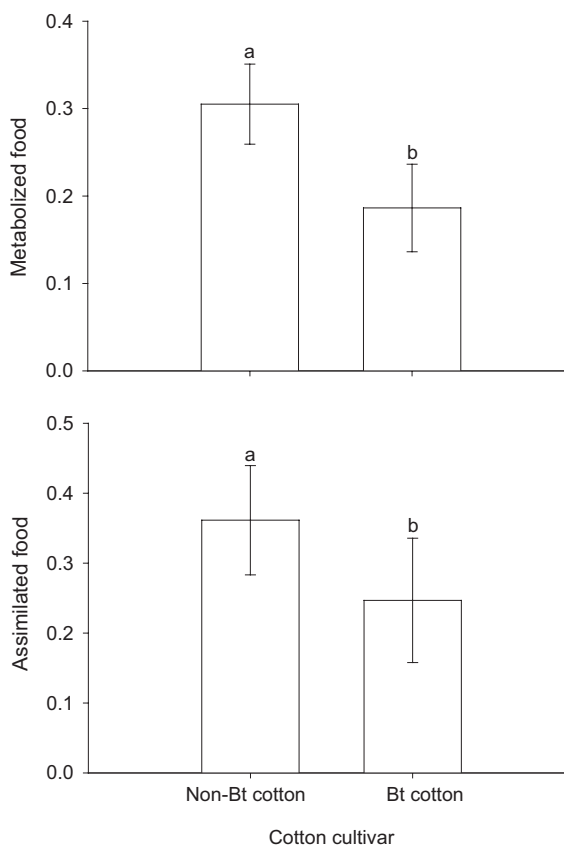


Figure 2 Mean (\pm SD) amount of food (g dry weight) metabolized ($F_{1,11} = 79.68$, $P = 0.0001$) and assimilated ($F_{1,11} = 81.48$, $P = 0.0001$) by fall armyworm larvae fed on non-Bt and Bt cotton. Different letters capping the bars within a panel indicate a significant difference between non-Bt and Bt cotton (Student–Newman–Keuls test: $P < 0.05$).

Bt-cotton leaves than for larvae that fed on non-transgenic cotton leaves (Table 1).

Discussion

Cry toxins from *B. thuringiensis* are now well known for their specific activities against insect species of the order of the Lepidoptera (Polanczyk & Alves, 2003; Ma et al., 2006). Several studies carried out under field conditions have shown that Bt cotton is highly effective as a pesticide to control the larvae of *Heliothis virescens* (Fabricius), *Helicoverpa zea* (Boddie), and other lepidopteran pests, inducing a drop in pest population densities in cotton fields (Shelton et al., 2002; Wu et al., 2003). According to Schnepf et al. (1998) and Wang (2008), Bt toxins act on the microvilli of columnar cells in the midgut epithelium of susceptible Lepidoptera. Inside the peritrophic membrane, protoxins are broken down by proteinases to produce toxic polypeptides that cross the membrane and then combine with specific receptors on the microvilli of the columnar cells of the intestine. This triggers a series of events, leading to osmotic imbalance in the epithelial cells and cell lysis, which enables entry of the toxins into the hemocoel, causing death by starvation and septicemia. Based on the results of our study, larvae of *S. frugiperda* fed on non-transgenic cotton leaves exhibit greater survivorship (96.7%) than those fed on Bt-cotton leaves (74.1%). However, the specific action of endotoxins synthesized from Cry1Ac genes was not sufficiently lethal to *S. frugiperda* larvae (25.9%) for controlling populations of this pest; this was also found in similar studies by Luttrell et al. (1999), Stewart et al. (2001), and Adamczyk & Gore (2004). The duration of the *S. frugiperda* larval phase was affected by cotton cultivar. It was longer for larvae fed on Bt-cotton leaves than for those fed on non-transgenic cotton leaves. *Spodoptera frugiperda* larval development (22.2 days) increased by 5.7% on a diet of Bt-cotton leaves compared to larvae fed on non-transgenic cotton leaves (21.0 days). Other studies reported that *S. frugiperda* survived on Bt-cotton leaves expressing the toxin Cry1Ac, although the larvae were smaller and the larval period prolonged (Adamczyk & Sumerford, 2001; Ashfaq et al., 2001; Stewart et al., 2001). With regard to pupal weight of *S. frugiperda*, larvae fed on Bt-cotton leaves (0.042 g) produced lighter pupae than those fed on non-transgenic cotton leaves (0.061 g).

Larvae fed on Bt-cotton leaves had a lower food intake and produced less feces than those fed on non-transgenic cotton leaves. However, weight gain was similar irrespective of the food source (transgenic or non-transgenic). Food quality is the main factor determining the utilization of food in many plant-feeding insects (Hodar et al., 2002),

because host plant components directly affect the plant-feeders' intake (Awmack & Leather, 2002).

The amount of food assimilated (food ingested and utilized by the insect for conversion into body matter and/or energy for metabolism) and the amount of food metabolized (food utilized in the form of metabolic energy) were lower in larvae fed on Bt-cotton leaves than in those fed on non-transgenic cotton leaves. This decrease could be because of Bt δ -endotoxin target cells of the midgut epithelium, which are important for enzyme production and nutrient absorption (Boucias & Pendland, 1998). On the other hand, Bt toxin reduces the absorption of amino acids and glucose (Fast & Angus, 1965; Gringorten, 2001).

The cotton cultivar significantly affected all nutritional indices for larval food consumption and utilization (RGR, RCR, RMR, AD, ECD, ECI, and MC): the figures were lower for larvae fed on Bt-cotton leaves than for larvae that fed on non-transgenic cotton leaves. The decrease in ECD (24.9% on non-Bt cotton, 15.9% on Bt cotton) could be because of allocation of energy for regeneration of the midgut epithelium damaged by δ -endotoxin (Luthy & Wolfersberger, 2000), rather than larval growth. Chen et al. (2005) reported that the efficiency of conversion of digested food and of ingested food of *Helicoverpa armigera* (Hübner) is significantly reduced when fed Bt cotton. Later instars use more food in the production of body matter and less in energy production, contributing to a higher ECD. Larvae need energy as they develop not only for maintenance but also for moving.

The Bt-cotton cultivar led to a drop in *S. frugiperda* food consumption, causing a drop in feces production, food metabolized, and food assimilated. However, we observed that the weight gain of larvae fed on Bt-cotton leaves was similar to that of larvae fed on non-transgenic cotton leaves. These results show that, although *S. frugiperda* is not considered a target pest for the toxins synthesized from the Cry1Ac gene, the presence of these toxins causes significant physiological alterations in *S. frugiperda* larvae.

Cotton fall armyworm is a major pest in several crops in South America, Central America, Mexico, and Southern USA (Ortega, 1987). The failure to control this pest by chemical means has led researchers to lay emphasis on the use of natural enemies in integrated management programs. Although *S. frugiperda* larvae show low susceptibility to Bt proteins, it could ingest these proteins from the plant and pass them on to higher trophic levels and interfere with established food chains (Torres et al., 2006).

These results associated with a drop in nutritional indices lead us to assume that the Bt-cotton cultivar is of low nutritional value for immature *S. frugiperda*. It causes a drop in RCR and therefore in the insect's RGR. Food

bioavailability has a direct impact on the insect's growth and development rates, so that the low growth rate of *S. frugiperda* is a result of a deficiency in the assimilation and subsequent conversion of nutrients into body matter (Slansky & Scriber, 1985). The drop in RCR is owing to the presence of allelochemical and toxic compounds in the food substrate, promoting phago-inhibitory responses (Scriber & Slansky, 1981). The presence of Bt endotoxins in the insect's digestive tract can lead to partial midgut paralysis, resulting in a drop in Bt leaf consumption (Prütz & Dettner, 2004). Approximate digestibility, an index that expresses the percentage of food effectively assimilated, was significantly lower for *S. frugiperda* larvae fed on Bt cotton. This shows that Bt δ -endotoxin affects AD. According to Boucias & Pendland (1998), Bt δ -endotoxin targets cells of the midgut epithelium, which are important for enzyme production and nutrient absorption. These low AD index values indicate that the food is not being retained for long in the larva's intestine. Rapid transition of food through the insect's digestive tract reduces the interaction of proteolytic enzymes on the food ball, causing a drop in active toxins in the gut lumen (Dinglasan et al., 2009). Larvae of *S. frugiperda* fed on Bt-cotton leaves exhibited lower ECI and ECD indices than those fed on non-transgenic cotton leaves. However, as less food is consumed, the organism develops physiological mechanisms that promote nutrient absorption and body matter assimilation, promoting growth (Waldbauer, 1968), and thus enabling the pupa to form, leading to functional adults with the capacity to reproduce (Bakker, 1961; Slansky & Scriber, 1985). The prolongation of the larval phase and the increased mortality caused by poor nutritional quality and/or the presence of toxins in the host plant means that the insect pest is exposed to its natural enemies for longer (Slansky, 1990). Analyzing this from an ecological viewpoint, the additional cost of nutritional compensation includes increased larval vulnerability to biotic factors (parasitoids, predators, and pathogens), as well as increased risk of exposure to toxins (Clancy & Price, 1987; Leather & Walsh, 1993; Boots, 2000). Thus, the results of this study are of primary importance to integrated pest management programs as a basis for developing management strategies for *S. frugiperda* in Bt cotton.

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