

Survival and nutritional indexes of *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) maintained in *Bt* maize for five generations

Luciana Barboza Silva¹, Kellen Maggioni¹, Raimundo Henrique Ferreira¹, Alexandre Faria Silva¹, Bruno Ettore Pavan², Gleidyane Novais Lopes¹

¹ Universidade Federal do Piauí, Campus Professora Cinobelina Elvas, Bom Jesus, PI, Brasil. E-mail: lubarbosabio@hotmail.com (ORCID: 0000-0002-7127-600X); kellenmaggioni@hotmail.com (ORCID: 0000-0002-7067-8111); raimundoagro117@gmail.com (ORCID: 0000-0003-4008-0810); afsilva9@hotmail.com (ORCID: 0000-0002-6964-6948); gnlopesm@hotmail.com (ORCID: 0000-0002-1455-3760)

² Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Engenharia de Ilha Solteira, Ilha Solteira, SP, Brasil. E-mail: brunoe.pavan@gmail.com (ORCID: 0000-0002-6487-5135)

ABSTRACT: The selection pressure generated by the incorrect use of *Bt* maize might result in *Spodoptera frugiperda* individuals resistant to the toxins synthesized by the plant. In this study was evaluated the nutritional parameters of *Spodoptera frugiperda* submitted to the maize that synthesize the toxins Cry1F, Cry1A105-Cry2Ab2 and Vip3Aa20, during five subsequent generations of selection pressure. The caterpillars were submitted to the treatments: Non-*Bt* maize (CONV), Cry1F (*Bt*1), Cry1A105/Cry2Ab (*Bt*2) and Vip3Aa20 (VIP1, VIP2 and VIP3) and exposed for a period of four days in each cycle, for five successive generations. The larval survival of *S. frugiperda* was evaluated in five generations. The consumed leaf area was quantified in the second generation and the nutritional indexes in the latter ones. The results indicate that caterpillars of *S. frugiperda* from VIPs lineage presented the lowest percentage of survival, with a high Metabolic Cost and a considerable efficiency reduction in the conversion of the digested and ingested food. The lineage maintained in VIP2 showed the lowest leaf consumption (83.13%). However, the action of Vip3Aa20 toxin on *S. frugiperda* resulted in a higher Metabolic Cost in reduction of leaf area consumption. However, from the third generation on there is a survival of insects exposed to *Bt* toxins, suggesting the surviving potential of this species in *Bt* maize subjected to continuous exposure to this technology.

Key words: *Bt* resistance; Cry toxins; fall armyworm; nutritional indexes; Vip3Aa20

Sobrevivência e índices nutricionais de *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) mantida em milho *Bt* por cinco gerações

RESUMO: A pressão de seleção gerada pelo uso incorreto do milho *Bt* pode resultar em indivíduos de *Spodoptera frugiperda* resistentes às toxinas expressadas pela planta. Neste trabalho foi determinado os parâmetros nutricionais de *Spodoptera frugiperda* submetida aos milhos que sintetizam as toxinas Cry1F, Cry1A105-Cry2Ab2 e Vip3Aa20, durante cinco gerações. As lagartas foram submetidas aos tratamentos: Milho não-*Bt* (CONV), Cry1F (*Bt*1), Cry1A105/Cry2Ab (*Bt*2) e Vip3Aa20 (VIP1, VIP2 e VIP3) e expostas por um período de quatro dias em cada ciclo, por cinco gerações sucessivas. Foi avaliada a sobrevivência larval de *S. frugiperda* em cinco gerações. A área foliar consumida foi quantificada na segunda geração e nas posteriores foram determinados os índices nutricionais. As lagartas de *S. frugiperda*, da geração mantida no milho VIPs, apresentaram menor porcentagem de sobrevivência, com um alto Custo Metabólico e considerável redução na Eficiência na conversão do alimento digerido e Eficiência na conversão do alimento ingerido. A geração mantida no Híbrido VIP2 apresentou o menor consumo foliar (83,13%). Contudo a ação da toxina Vip3Aa20 sobre *S. frugiperda* resultou no maior Custo Metabólico em redução no consumo de área foliar. Contudo a partir da terceira geração observa-se uma sobrevivência de insetos expostos as toxinas *Bt*, sugerindo o potencial desta espécie em sobreviver aos milhos *Bt* quando submetidas a exposição contínua a esta tecnologia.

Palavras-chave: resistência *Bt*; toxinas Cry; lagartas-do-cartucho; índices nutricionais; Vip3Aa20

Introduction

A Bt maize contains one or more genes from the entomopathogenic bacterium *Bacillus thuringiensis*, which synthesizes toxins against the crop main pest, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), popularly known as the fall armyworm. Due to the high control rate provided by the Bt technology, farmers grow Bt maize throughout the year, without adopting adequate refuge areas (Storer et al., 2010; Farias et al., 2016).

With the rising use of Bt maize, there is an increase in selection pressure on *S. frugiperda*, resulting in caterpillars resistant to the toxins synthesized by Bt maize (Storer et al. 2010, Farias et al., 2016, Bernardi et al., 2017). This resistance may be associated with an alteration in the toxin site of action (due to an adaptive response), and/or be linked to the stage of development or even the attacked part of the plant, whereas this site synthesizes a low amount of the toxin (Wang et al., 2004; Santos-Amaya et al., 2017; Yinghua et al., 2017).

Insects resistant to Bt toxins, such as Cry1F toxin, may display alterations in several biological parameters, such as the development time from egg to adult and also reproduction, with an adaptive metabolic cost in response to Cry toxins resistance being noted (Bernardi et al., 2017).

The metabolic cost can be estimated from quantitative nutritional studies, with nutritional indexes being related to the amount of used food, digested, assimilated, metabolized and converted into body material (Scriber & Slansky Jr, 1981). The efficiency in the conversion of digested and ingested food are the main nutritional indexes related to the metabolic cost of *S. frugiperda* to Bt maize (Yinghua et al., 2017). In Bt cotton that synthesizes the Cry1Ac toxin, the efficiency in the conversion of the digested and ingested food for *S. frugiperda* was reduced by 9.05 and 8.24%, respectively in relation to the control group (Ramalho et al., 2011).

Reports suggest high evolution risk of the *S. frugiperda* resistance to pyramidal maize and to the Vip3Aa20 event (Bernardi et al., 2015a; Bernardi et al, 2017). The Bt toxins present in plant leaves can affect the amount of food ingested and converted into body matter, as well as increasing energy expense during metabolism (Ramalho et al., 2011). Thus, the aim of this study was to evaluate the survival and nutritional indexes of *Spodoptera frugiperda* fed with the maize hybrids that synthesize the toxins Cry1F, Cry1A105-Cry2Ab2 and Vip3Aa20 for five generations.

Materials and Methods

Study site

The study was conducted in the Phytotechnology Laboratory, Federal University of Piauí, Professor Cinobelina Elvas Campus, Bom Jesus, PI. The experiment was conducted from November 2012 to October 2013.

Collection of the caterpillars

The insects were collected in conventional and transgenic maize crops (in order to obtain a population with greater

genetic variability) at commercial planting areas in the Upper Middle Gurguéia region, Piauí, during the 2012/13 harvest. The collected caterpillars were individually packed in 100 mL plastic containers with a lid.

Creation maintenance

The *S. frugiperda* caterpillars were sustained with an artificial diet adapted from Kasten Júnior et al. (1978) in a controlled environment (25±2°C; 60±25%RH; 12:12LD). The insects were kept for three generations in order to increase the genetic variability of the population. Thereby, a population with a homogeneous stage of development, with a sufficient number of caterpillars to assemble the bioassays, was obtained in this said generation. Neonates caterpillars (<24h old) were individualized and transferred to 100 mL plastic containers supplied with artificial diet until they reached the pupal stage. The pupae were then transferred to PVC cages (40 cm h x 30 cm Ø), internally covered with sulphite paper sheets, for oviposition. The adults were fed a solution composed by distilled water and honey (10%), and kept at a temperature of 25°C ± 2°C. The egg masses were collected and stored in plastic bags, and kept in controlled environment until they hatched.

Cultivation of maize hybrids

The maize hybrids and their respective toxins used in the experiments were: CONV – non-Bt hybrid (DuPont Pioneer 30F53); Bt1 – Cry1F (DuPont Pioneer 30F53H); Bt2 – Cry1A105-Cry2Ab2 (Syngenta Ag7088); VIP1 – Vip3Aa20 (Syngenta 7205); VIP2 – Vip3Aa20 (Syngenta PENTA); VIP3 – Vip3Aa20 (Syngenta 8A 98). The CONV and Bt1 hybrids are isogenic (same hybrid, but with the introduction of the toxin into Bt1), and the VIPs all have the same insecticidal toxins, in different hybrids.

The selection pressure process was performed using leaves from Bt maize hybrids between 20 and 40 days after the emergence, from plants kept inside a greenhouse in pots with 4 Kg of corrected and fertilized soil, as recommended for the crop (Sousa & Lobato, 2004), without exposure to insecticides. The leaves were collected, sanitized (1% sanitary water solution for 10 minutes), cut and made available for feeding the caterpillars for a period of four days. Confirmation of leaf protein expression was performed by immunodetection tests of the interest proteins using strips from the QuickStix Kit for Vip3A, Cry1F and Cry1A according to instructions of the manufacturer (EnviroLogix Inc. 2013).

Obtaining the lineages of *Spodoptera frugiperda*

The breeding of the collected insects was held in order to obtain a base population with greater genetic variability. The progenies of these breeds were divided into sub-populations or lineages. The caterpillars remained on artificial diet until the third instar, and later were fed maize leaves, where each hybrid was considered as a treatment and as corresponding to a lineage. One of the lineages was kept in the absence of selection pressure in conventional maize leaves. The others

were submitted to the transgenic events containing the proteins Cry1F, Cry1A105/Cry2Ab and VIP3AA20. The caterpillars were exposed for four days in each generation, for five generations. The principle of low selection pressure proposed by Roush & McKenzie (1987) was adopted, where the high dose makes the expression of the resistance effectively or functionally recessive, eliminating the heterozygous individuals, with the low being able to cause an effectively dominant resistance.

Surviving insects, after this period, were kept on an artificial diet in a controlled environment as previously described, until they completed the cycle.

Evaluated parameters

The nutritional indexes from the different lineages of *S. frugiperda* were determined using the fresh weight of the leaves, caterpillars and feces of the insects, where they fasted for 15 hours prior to that. Afterwards, the caterpillars were weighed and fed with the leaves of the previously weighed hybrids. After four days, another weight of the caterpillars, leaves and feces was done, following the proposed methodology by Waldbauer (1968), modified by Scriber & Slansky Jr (1981). Eight replications (10 caterpillars/replicate) of each lineage were used, kept for four days in maize leaves. The calculated parameters were: T = duration of the feeding period (days); Af = weight of food given to the insect (g); Ar = weight of the leftover food given to the insect (g), after T; F = weight of produced feces (g) during T; b = caterpillars weight gain (g) during T; B = caterpillars mean weight (g) during T; I = weight of the ingested food (g) during T; I - F = assimilated food (g) during T; M = (I - F) - B = metabolized food during the feeding period. For the weights measurement, a precision analytical balance was employed. The calculated consumption indexes were:

- Relative consumption rate (RCR) = $I/B \cdot T$;
- RGR (Relative growth rate) = $b/B \cdot T$;
- RMR (Relative metabolic rate) = $M/B \cdot T$;
- AD (approximate digestibility) = $I - F / I \cdot 100$;
- ECI (Conversion efficiency of ingested food) = $b / I \cdot 100$;
- ECD (Conversion efficiency of digested food) = $b / I - F \cdot 100$;
- CM (metabolic cost) = $100 - ECD$.

The leaf area was estimated using the LI - 3.100C Area Meter (LI-COR® Biosciences) equipment, in the second generation of individuals. Fresh leaves were cut, measured and offered to the caterpillars, and at the end of the four-day experiment time, the leaves were measured again, where the difference between the readings corresponded to the leaf area consumed by the caterpillar.

Statistical analysis

The bioassay was conducted in a completely randomized design (DIC) consisting of five treatments and eight replicates each. Data regarding the mortality were transformed to $\sqrt{x} + 0.5$ and subjected to analysis of variance (Proc GLM). When found a significant difference, the results were submitted to the Duncan mean comparison test, and the accumulated

mortality index per generation adjusted with eight replicates for each treatment within the five generations, with 80 insects per treatment (10 caterpillars/replicate).

The nutritional indexes were transformed into arch of x cotangent, in radians, and subsequently subjected to the individual analysis of variance (per generation) (Proc GLM), where the variances were compared by Fisher test and, after homogeneity verification of the residual variances, the joint analysis was held. When significant difference was detected for the interaction, the unfolding within and between generations was performed using Duncan mean test.

The Pearson correlation coefficient was determined using nutritional indexes within each generation. In the second generation, the leaf area consumed by the caterpillars was evaluated, where 32 replicates were held for each hybrid. Analyzes were performed by PROC GLM, and the Duncan means comparison test. Statistical analyzes were performed with the SAS program (SAS, 2002).

Results

Caterpillars fed with *Bt* maize hybrids had larval survival reduced over the five generations. The mean survival of *S. frugiperda* within each generation when fed with the hybrids CONV, Bt1, Bt2, VIP1, VIP2, VIP3 showed a statistical difference (Figure 1).

The survival percentage of *S. frugiperda* caterpillars kept without selection pressure and fed with non-*Bt* maize remained relatively constant over the generations. Among the genetically modified hybrids, survival was highest in *Bt* hybrids, up to the third generation, when compared to VIP hybrids. From the third generation on, the survival was rising in all *Bt* hybrids. From the fourth generation of breeding on, there was a survival increase in the Bt2 and VIP1 lineages, while in the others we observed a slight decrease. VIP2 and VIP3 reduced insect survival within the evaluated generations by 34.38% and 28.13%, respectively.

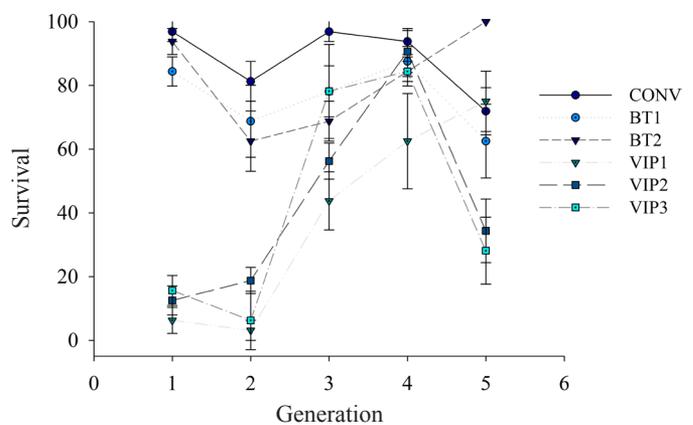


Figure 1. Survival of *S. frugiperda* in five generations during selection pressure. (F = 6.31, G.L. = 18, 208, P = 0.01, C.V. 42.97%). CONV – (non-*Bt* hybrid); Bt1 – (Cry1F); Bt2 – (Cry1A105-Cry2Ab2); VIP1 – (Vip3Aa20); VIP2 – (Vip3Aa20); VIP3 – (Vip3Aa20).

Results regarding nutritional indexes of *S. frugiperda* showed statistical significance (Table 1). Due to problems regarding vigor and germination of the Bt1 hybrid, its nutritional indexes were not estimated. The metabolized food (M) was lower in the third and fifth generations of *S. frugiperda* in CONV and VIP lineages populations, respectively. Caterpillars exposed to the VIP3 hybrid presented a relative consumption rate (RCR) similar to the conventional lineage insects. In the fifth generation, the insects kept in VIP1 hybrid had the lowest relative Consumption Rate (Table 1). Throughout the generations, the relative consumption rate remained similar to that displayed in the third generation by the CONV lineage caterpillars.

Treatments caused an alteration in the relative growth rate (RGR). Within the third generation, only the CONV lineage caterpillars presented growth when compared to the others. In the fifth generation, the CONV and Bt2 lineages caterpillars also maintained the growth. These results suggest a nutritional adaptation by the caterpillar to the Cry1A105-Cry2Ab2 toxin over the generations. Regarding the relative metabolic rate (RMR), the caterpillars kept in

the CONV and VIP1 hybrids had the lowest metabolic rates in the third and fifth generations, respectively. In the fifth generation, the caterpillars from VIP2 and VIP3 lineages presented a high relative consumption with a decrease in the relative growth rate and reduced efficiency in the conversion of the ingested and digested food, resulting in a high metabolic cost.

According to the Pearson correlation coefficients (Table 2), we can note that in the third generation, there is negative correlation between the approximate digestibility and the growth rate and relative consumption indexes, with the RCR showing a positive correlation with RGR, ECD and ECI. Where the conversion efficiency of the ingested food present a positive correlation with RGR and ECD. For the fifth generation, we observed that the lower the RGR, ECI and ECD values, the higher the metabolic cost is (Table 2). We can also observe that the CONV variety had the largest consumption of leaf area in the second generation (Figure 2). Contrasting to this, the VIP hybrids had a greater reduction in leaf consumption, with emphasis the VIP1 and VIP2 hybrids, with about 80% less consumption than the control group.

Table 1. Nutritional levels (\pm EP) of *Spodoptera frugiperda* fed for 4 days with maize hybrids leaves displaying different toxins.

Hybrids	3 rd Generation		5 th Generation	
	Metabolized food (M)		Approximate digestibility (AD)	
CONV	-0.006 \pm 0.001 Ba	0.141 \pm 0.02 Ab	49.1 \pm 4.18 Ba	81.73 \pm 3.66 Ab
Bt2	0.032 \pm 0.01 Ab	0,086 \pm 0.007 Aa	76.7 \pm 6.41 Aa	82.04 \pm 3.08 Aa
VIP1	0.053 \pm 0.02 Aa	0,054 \pm 0.008 Ba	77.7 \pm 5.64 Aa	89.46 \pm 5.1 Aa
VIP2	0.080 \pm 0.03 Aa	0,105 \pm 0.004 Aa	86.1 \pm 4.92 Aa	99.88 \pm 0.12 Aa
VIP3	0.047 \pm 0.004 Aa	0,117 \pm 0.014 Ab	87.6 \pm 2.95 Aa	98.81 \pm 0.27 Aa
F		5**		6**
C.V.%		3.39		27.34
Relative consumption rate (RCR)		Conversion efficiency of ingested food (ECI)		
CONV	3.067 \pm 0.12 Aa	3.16 \pm 0.63 Ba	5.234 \pm 0.74 Aa	8.403 \pm 1.19 Aa
Bt2	1.305 \pm 0.16 Ba	4.54 \pm 1.61 Bb	-5.703 \pm 3.28 Ba	5.795 \pm 1.63 Ab
VIP1	1.541 \pm 0.29 Ba	1.78 \pm 0.52Cb	-11.247 \pm 2.50 Ba	-4.884 \pm 0.85 Ba
VIP2	1.385 \pm 0.39 Ba	11.32 \pm 1.95 Aa	-14.114 \pm 2.38 Ba	-1.151 \pm 0.31 Ba
VIP3	2.389 \pm 0.38 Aa	9.43 \pm 1.24 Aa	-2.342 \pm 0.51 Ba	-1.346 \pm 0.41 Ba
F		11.90**		4.39**
C.V.%		39.80		33.42
Relative growth rate (RGR)		Conversion efficiency of digested food (ECD)		
CONV	0.160 \pm 0.02 Aa	0.195 \pm 0.02 Aa	12,204 \pm 2.32 Aa	11.021 \pm 1.86 Aa
Bt2	-0.034 \pm 0.04 Ca	0.157 \pm 0.05 Ab	-4.026 \pm 5.26 Ba	7.591 \pm 2.03 Ab
VIP1	-0.123 \pm 0.02 Ba	-0.067 \pm 0.01 Ba	-15.703 \pm 3.52 Ca	-6.241 \pm 1.67 Ba
VIP2	-0.140 \pm 0.01 Ba	-0.095 \pm 0.01 Ba	-16.481 \pm 2.41 Ca	-1.153 \pm 0.31 Ba
VIP3	-0.053 \pm 0.01 BCa	-0.107 \pm 0.03 Ba	-2.541 \pm 0.54 Ba	-1.361 \pm 0.42 Ba
F		46.2**		4.5**
C.V.%		5.64		34.19
Relative metabolic rate (RMR)		Metabolic cost (CM)		
CONV	-0.069 \pm 0.26 Ca	2.266 \pm 0.64 Ab	87.796 \pm 2.32 Ca	88.979 \pm 1.86 Ba
Bt2	0.562 \pm 0.16 Ba	3.539 \pm 1.68 Ab	104.026 \pm 5.26 Ba	92.409 \pm 2.04 Ba
VIP1	1.044 \pm 0.32 Aba	1.503 \pm 0.56 Ba	115.703 \pm 3.52 Aa	106.241 \pm 1.67 Aa
VIP2	1.146 \pm 0.42 Aa	11.30 \pm 1.95 Ab	116.481 \pm 2.42 Aa	101.153 \pm 0.31 ABb
VIP3	1.806 \pm 0.25 Aa	9.228 \pm 1.24 Ab	102.541 \pm 0.54 Aba	101.361 \pm 0.42 Aba
F		4.62**		2**
C.V.%		51.51		8.67

**Significant at 1% probability by F test. Means followed by the same uppercase letter in the column and lowercase in the line do not differ significantly from each other by the Duncan test at 5% probability. RCR, RGR, RMR are in g/g/day; and AD, ECI, ECD and CM in %. SE: Mean standard error.

Table 2. Pearson correlation coefficients for the traits Metabolized Food (M), Approximate Digestibility (AD), Relative Growth Rate (RGR), Relative Metabolic Rate (RMR), Relative Consumption Rate (RCR), Conversion Efficiency of Ingested Food (ECI), Conversion Efficiency of Digested Food (ECD) and Metabolic Cost (CM) of *Spodoptera frugiperda* fed with maize hybrids.

Indexes	M	AD	RCR	ECI	RGR	ECD	RMR
3 rd Generation							
AD	0.90 ^{ns}						
RCR	-0.92 ^{ns}	-0.73 ^{ns}					
ECI	-0.93 ^{ns}	-0.73 ^{ns}	0.99**				
RGR	-0.77 ^{ns}	0.46 ^{ns}	0.99*	0.99*			
ECD	-0.81 ^{ns}	-0.54 ^{ns}	0.96*	0.96*	0.99 ^{ns}		
RMR	-0.91 ^{ns}	-0.96*	0.83 ^{ns}	0.82 ^{ns}	0.95 ^{ns}	0.67 ^{ns}	
CM	0.67 ^{ns}	-0.57 ^{ns}	-0.99 ^{ns}	-0.98 ^{ns}	-0.99 ^{ns}	-1.00**	-0.98 ^{ns}
5 th Generation							
AD	-0.004 ^{ns}						
RCR	0.33 ^{ns}	0.83 ^{ns}					
ECI	0.62 ^{ns}	-0.71 ^{ns}	-0.25 ^{ns}				
RGR	0.33 ^{ns}	-0.92*	-0.56 ^{ns}	0.93*			
ECD	0.64 ^{ns}	-0.70 ^{ns}	-0.23 ^{ns}	0.99**	0.92*		
RMR	0.29 ^{ns}	0.87 ^{ns}	0.99**	-0.32 ^{ns}	-0.62 ^{ns}	-0.30 ^{ns}	
CM	-0.64 ^{ns}	0.70 ^{ns}	0.23 ^{ns}	-0.99**	-0.92*	-1.00**	0.30 ^{ns}

Not significant (ns). Significant at 1 (**) and at 5% (*) probability by the F test, respectively.

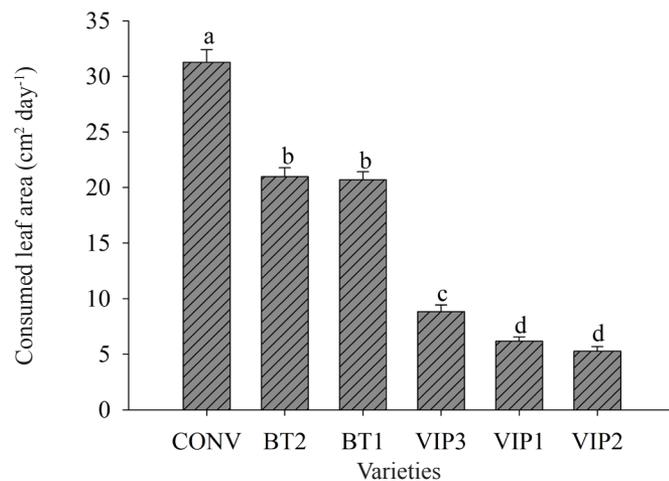


Figure 2. Leaf area consumed by *Spodoptera frugiperda* caterpillars in the second generation fed with *Bt* maize leaves. Means followed by the same letter do not differ significantly from each other by the Duncan test ($p < 0.01$).

Discussion

The selection pressure on *S. frugiperda* brought by the continuous use of *Bt* maize, without having a refuge crop, can condition the selection of resistant insects, resulting in a reduced efficiency of the technology employed (Storer et al., 2010; Farias et al., 2016). Evaluation by successive generations, in this study, resulted in a greater survival of individuals after five generations. High survival was also verified after ingestion of the Cry toxins, although having a metabolic cost (CM) and a marked reduction in leaf consumption.

The recorded survival percentage since the first generation indicates that the fall armyworm already had some response variability to the Cry1F and Cry1A105-Cry2Ab2 toxins expressed in the studied maize hybrids, and consequently no change in the insect behavior was observed. Reports

of populations resistant to the Cry1F toxin are already documented in the literature (Storer et al., 2010). Survival of at least 35% of *S. frugiperda* in the *Bt* Cry1F maize (Bernardi et al., 2015b; Farias et al., 2016; Yang et al., 2016).

There was a reduction in the control efficiency in pyramided (Cry1A105-Cry2Ab2) and non-pyramided (Cry1F) events. This finding contradicts the premise that transgenic crops that have two or more toxins would be more effective in delaying the resistance evolution when compared to crops with only one toxin (Tabashnik & Gould, 2012), due to the occurrence of cross-resistance (Bernardi et al., 2015b). Resistant insects possess the ability to eliminate *Bt* toxins from their intestine, in addition to altering the way Cry toxins act, invalidating the toxic action (Pérez-Hedo et al., 2012).

The significant increase in the survival of caterpillars exposed to hybrids with Vip3a20 technology within three generations corroborates with the obtained results by Miraldo et al. (2016), where the exposure of *S. frugiperda* to Vip3Aa20 maize resulted in the survival of around 20% of caterpillars. Bernardi et al. (2015a) cite that, due to technology misuse, the fall armyworm may develop resistance to Vip3Aa20 toxins. However, the survival percentages of caterpillars exposed to the used transgenic maize hybrids may be tied to the original crops, where the population used in this study was in the third generation and the insects can maintain resistance in environments without selection pressure for up to seven generations (Santos-Amaya et al., 2017).

The caterpillars exposed to *Bt* hybrids display altered biological characteristics when compared to insects kept in non-*Bt* maize hybrids (Bernardi et al., 2017). The nutritional indexes of lineages kept on transgenic maize leaves indicate metabolic cost, with the caterpillars kept on VIP maize (Vip3Aa20 toxin) showing more sensitiveness to toxin action when compared to the insects kept in maize with Cry1A105-Cry2Ab2 pyramidal events. Lineages of *S. frugiperda* exposed to the Cry1F toxin did not present aptitude costs relevant

to the development in question (Horikoshi et al., 2016). We verified in this study that the conversion of ingested, digested (ECI, ECD) and relative growth (RGR) showed a strong negative correlation with the metabolic cost (CM). Usually, organisms exposed to *Bt* maize shows high metabolic cost in direct response to the toxins action (Ramalho et al., 2011; Cataño et al., 2014). The estimated nutritional indexes were affected by the evaluated toxins action, as well as the obtained results with the exposure of *S. frugiperda* to *Bt* cotton (Ramalho et al., 2011; Cataño et al., 2014).

The metabolic cost of caterpillars grown on VIP maize (Vip3Aa20 event) was similar to *S. litura* fed with *Bt* maize (Cry1Ab). In this case, there was high consumption without weight gain, where we noted high levels of digested feed (AD) and relative consumption rate (RCR) and low feed conversion efficiency (ECI), digested feed conversion (ECD) and relative growth rate (RGR), indicating an energy shift from biomass production to toxin detoxification (Yinghua et al., 2017).

The changes in metabolized feed (M), relative growth rate (RGR), relative metabolic rate (RMI) and relative consumption rate (RCR) are the result of a deficiency in assimilation and subsequent conversion of nutrients into body material and the inactivation of *Bt* toxins (Ramalho et al., 2011; Yinghua et al., 2017). In virtue of the presence of toxic substances without interference in palatability, caterpillar intoxication occurs due to the action of Cry toxins, affecting the usage of ingested food (Scriber & Slansky, 1981; Jiang et al., 2013). Thus, the ability of an organism to convert nutrients and protein will influence positively its growth (Sogbesan & Ugwumba, 2008).

The lower values of Approximate Digestibility (AD) obtained in *S. frugiperda* not exposed to *Bt* toxins may be related to fast food digestibility in the intestine of the insect (Ramalho et al., 2011). The rapid transition of food through the digestive tract of the insect reduces the interaction of proteolytic enzymes in the food substrate, causing a decrease in the active toxins in the intestinal lumen (Dinglasan et al., 2009). On the other hand, caterpillars fed with *Bt* maize, mainly those that synthesize the Vip3Aa20 toxin, presented high values of approximate digestibility, indicating that the toxin inactivation affects the food digestibility, since the Cry toxins action in the intestine of the insects alter the activity of the digestive enzymes (Yinghua et al., 2017).

Changes in the conversion efficiency of ingested and digested food may be related to the presence of Vip3Aa20, as the action of *Bt* toxins reduces the caterpillars ability in assimilating the nutrients (Cataño et al., 2014), or the resulting energy from assimilation of food is being used in the regeneration of midgut epithelium, damaged by the Cry toxins action (Lüthy & Wolfersberger, 2000). Caterpillars grown with VIP maize (Vip3Aa20 event) presented the highest values of metabolic cost, probably due to an inefficient conversion of biomass with low efficiency values in the conversion of ingested and digested food (Cataño et al., 2014).

When comparing the generations, in the third generation the CONV lineage caterpillars and in the fifth generation the Bt2 lineage (Cry1A105-Cry2Ab2 event) presented an

increase in the rates of conversion efficiency of the ingested and digested food. Thus, nutritional indexes (especially ECD and ECI) can be considered important within the parameters related to the survival of insects exposed to *Bt* maize.

CONV and VIP hybrids presented the highest and lowest leaf area consumed, respectively. The presence of Vip3Aa20 toxin in the digestive tract of the insect may have led to midgut partial paralysis, resulting in a decrease in leaf consumption and a considerable metabolic cost (Prütz & Dettner, 2004; Storer et al., 2010; Farias et al., 2016).

The Cry1F-Cry1A105-Cry2Ab2 pyramidal event caused a reduction of 55.14% in the leaf consumption of *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae), in relation to the control group (Bortolotto et al., 2015), results higher than those found in this study, where maize with two proteins (Cry1A105-Cry2Ab2) had a reduction of 32.93% in the consumed leaf area. A low response in the conversion of ingested and deferred food by the insects may result in a greater consumption of leaf area; however, further studies are necessary in order to consolidate this premise. The results contrast with the literature premises, that pyramidal events present a higher percentage of control, resistance prevention and lower leaf consumption (Bortolotto et al., 2015). Costa et al. (2006), observed a 28.92 cm².day⁻¹ consumption of *S. frugiperda* in leaves of conventional maize, compatible with the result found in this study.

According to the obtained results, it can be established that *Bt* maize hybrids containing one or more toxins affect the nutritional indexes and the consumed leaf area. In addition to providing subsidy for new studies regarding the activity of the detoxifying enzymes present in the intestines of insects for a better understanding of the *Bt* toxins toxic effects, studies of cross-resistance to other toxins, which will help in the selection of these for pyramiding in maize plants and/or for the rotation of maize hybrids expressing different toxins. As well as the genetic, biochemical and molecular characterization of lineage resistance, this may aid in the refinement of recommendations for the management of *S. frugiperda* resistance to transgenic maize with Cry1F, Cry1A105-Cry2Ab2 and Vip3Aa20.

Conclusion

Spodoptera frugiperda grown with Vip3Aa20 maize presents a survival percentage increase from the second generation onwards.

The relative growth rate, the conversion efficiency of the ingested and digested food present a negative correlation with the metabolic cost in populations of *S. frugiperda* maintained in *Bt* maize hybrids.

Vip3Aa20 maize carries the highest metabolic cost with reduction in leaf consumption of *Spodoptera frugiperda*.

Bt maize hybrids with the Cry1A105-Cry2Ab2 and Vip3Aa20 toxins present low survival percentages with changes in nutritional indexes and can be employed as an alternative in the *Spodoptera frugiperda* management.

Acknowledgements

We thank the DuPont Young Professor Grant program, the National Council for Scientific and Technological Development (CNPq) and the CAPES Foundation and Research Support Foundation of Piauí State (FAPEPI) for their financial support.

Literature Cited

- Bernardi, D.; Bernardi, O.; Horikoshi, R. J.; Salmeron, E.; Okuma, D. M.; Farias, J. R.; Nascimento, A.R.B.; Omoto, C. Selection and characterization of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistance to MON 89034× TC1507× NK603 maize technology. *Crop Protection*, v.94, p. 64-68, 2017. <https://doi.org/10.1016/j.cropro.2016.11.026>.
- Bernardi, D.; Salmeron, E.; Horikoshi, R. J.; Bernardi, O.; Dourado, P. M.; Carvalho, R. A.; Martinelli, S.; Head G.P.; Omoto, C. Cross-resistance between Cry1 proteins in fall armyworm (*Spodoptera frugiperda*) may affect the durability of current pyramided Bt maize hybrids in Brazil. *PLoS ONE*, v.10, n.10, e0140130, 2015b. <https://doi.org/10.1371/journal.pone.0140130>.
- Bernardi, O.; Bernardi, D.; Amado, D.; Sousa, R. S.; Fatoetto, J.; Medeiros, F. C. Conville, J.; Burd, T.; Omoto, C. Resistance risk assessment of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Diatraea saccharalis* (Lepidoptera: Crambidae) to Vip3Aa20 insecticidal protein expressed in corn. *Journal of Economic Entomology*, v.108, n.6, p.2711-2719, 2015a. <https://doi.org/10.1093/jee/tov219>.
- Bortolotto, O. C.; Bueno, A. D. F.; Queiroz, A. P. D.; Silva, G. V.; Barbosa, G. C. Larval development of *Spodoptera eridania* (Cramer) fed on leaves of Bt maize expressing Cry1F and Cry1F+ Cry1A. 105+ Cry2Ab2 proteins and its non-Bt isolate. *Revista Brasileira de Entomologia*, v.59, n.1, p.7-11, 2015. <https://doi.org/10.1016/j.rbe.2014.12.001>.
- Cataño, S. J. V.; Chalarca, J. R.; Cobo, N. C. M. Efecto de variedades de algodón genéticamente modificadas sobre larvas de *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae). *Acta Agronómica*, v.63, n.1, p.63, 2014. <https://doi.org/10.15446/acag.v63n1.38356>.
- Costa, M. A. G.; Grützmacher, A. D.; Zotti, M. J.; Härter, W. R.; Neves, M. B. Consumo foliar e preferência de *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) por cultivares de milho e sorgo. *Revista Brasileira de Agrociência*, v.12, n.4, p.415-421, 2006. <http://www.ufpel.tche.br/faem/agrociencia/v12n4/artigo06.pdf>. 18. Nov 2017.
- Dinglasan, R. R.; Devenport, M.; Florens, L.; Johnson, J. R.; McHugh, C. A.; Donnelly-Doman, M.; Carucci, D. J.; Yates, J. R.; Jacobs-Lorena M. The *Anopheles gambiae* adult midgut peritrophic matrix proteome. *Insect Biochemistry and Molecular Biology*, v. 39, n. 2, p. 125-134, 2009. <https://doi.org/10.1016/j.ibmb.2008.10.010.07>.
- Farias, J. R.; Andow, D. A.; Horikoshi, R. J.; Bernardi, D.; Ribeiro, R. D. S.; Nascimento, A. R.; Santos, A.C.; Omoto, C. Frequency of Cry1F resistance alleles in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. *Pest Management Science*, v.72, n.12, p.2295-2302, 2016. <https://doi.org/10.1002/ps.4274>.
- Horikoshi, R. J.; Bernardi, O.; Bernardi, D.; Okuma, D. M.; Farias, J. R.; Miraldo, L. L.; Amaral, F. S. A.; Omoto, C. Near-isogenic Cry1F-resistant strain of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to investigate fitness cost associated with resistance in Brazil. *Journal of Economic Entomology*, v.109, n.2, p. 854-859, 2016. <https://doi.org/10.1093/jee/tov387>.
- Jiang, X. F.; Chen, J.; Zhang, L.; Sappington, T. W.; Luo, L. Z. Increased long-flight activity triggered in beet armyworm by larval feeding on diet containing Cry1Ac protoxin. *PLoS ONE*, v. 8, n. 5, e63554, 2013. <https://doi.org/10.1371/journal.pone.0063554>.
- Kasten Júnior, P.; Precetti, A. A. C. M.; Parra, J. R. P. Dados biológicos comparativos de *Spodoptera frugiperda* (J.E.Smith, 1797) em duas dietas artificiais e substrato natural. *Revista Agricultura*, v.53, n. 1/2, p. 68-78, 1978. <http://agris.fao.org/agris-search/search.do?recordID=US201302850316>. 07 Abr. 2017.
- Lüthy, P.; Wolfersberger, M. G Pathogenesis of *Bacillus thuringiensis* toxins. In: Charles, J.-F.; Delécluse, A.; Nielsen-Le Roux, C. (Eds.). *Entomopathogenic bacteria: from laboratory to field application*. Dordrecht: Springer, 2000. p. 167-180. https://doi.org/10.1007/978-94-017-1429-7_9.
- Miraldo, L. L.; Bernardi, O.; Horikoshi, R. J.; Amaral, F. S.; Bernardi, D.; Omoto, C. Functional dominance of different aged larvae of Bt-resistant *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on transgenic maize expressing Vip3Aa20 protein. *Crop Protection*, v.88, p.65-71, 2016. <https://doi.org/10.1016/j.cropro.2016.06.004>.
- Pérez-Hedo, M.; López, C.; Albajes, R.; Eizaguirre, M. Low susceptibility of non-target Lepidopteran maize pests to the Bt protein Cry1Ab. *Bulletin of Entomological Research*, v.102, n.6, p.737-743, 2012. <https://doi.org/10.1017/S0007485312000351>.
- Prütz, G.; Dettner, K. Effect of Bt corn leaf suspension on food consumption by *Chilo partellus* and life history parameters of its parasitoid *Cotesia flavipes* under laboratory conditions. *Entomologia Experimentalis et Applicata*, v.111, n.3, p.179-187, 2004. <https://doi.org/10.1111/j.0013-8703.2004.00166.x>.
- Ramallo, F. S.; Azeredo, T. L.; Nascimento, A. R. B.; Fernandes, F. S.; Nascimento Júnior, J. L.; Malaquias, J.; Silva, C. A. D.; Zanúncio, J. C. Feeding of fall armyworm, *Spodoptera frugiperda*, on Bt transgenic cotton and its isolate. *Entomologia Experimentalis et Applicata*, v. 139, n. 3, p. 207-214, 2011. <https://doi.org/10.1111/j.1570-7458.2011.01121.x>.
- Roush, R. T.; McKenzie, J. A. Ecological genetics of insecticide and acaricide resistance. *Annual Review of Entomology*. v. 32, n. 1, p. 361-380, 1987. <https://doi.org/10.1146/annurev.en.32.010187.002045>.
- Santos-Amaya, O. F.; Tavares, C. S.; Rodrigues, J. V. C.; Campos, S. O.; Guedes, R. N. C.; Alves, A. P.; Pereira, E. J. G. Fitness costs and stability of Cry1Fa resistance in Brazilian populations of *Spodoptera frugiperda*. *Pest Management Science*, v 73, n.1, p.35-43, 2017. <https://doi.org/10.1002/ps.4312>.
- SAS Institute. SAS user's manual. Version 9.1. Cary: SAS Institute, 2002.
- Scriber, J. M.; Slansky Jr, F. The nutritional ecology of immature insects. *Annual Review of Entomology*, v.26, n.1, p.183-211, 1981. <https://doi.org/10.1146/annurev.en.26.010181.001151>. 07 Abr. 2017.

- Sogbesan, A. O.; Ugwumba, A. A. A. Nutritional evaluation of termite (*Macrotermes subhyalinus*) meal as animal protein supplements in the diets of *Heterobranchus longifilis* (Valenciennes, 1840) fingerlings. *Turkish Journal of Fisheries and Aquatic Sciences*, v.8, n.1, p.49–157, 2008. <http://www.trjfas.org/abstract.php?id=603>. 18. Nov 2017.
- Sousa, D. M. G.; Lobato, E. Cerrado: correção do solo e adubação. 2.ed. Planaltina: Embrapa Cerrados, 2004. 416p.
- Storer, N. P.; Babcock, J. M.; Schlenz, M.; Meade, T.; Thompson, G. D.; Bing, J. W.; Huckaba, R. M. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. *Journal of Economic Entomology*, v.103, n.4, p.1031-1038, 2010. <https://doi.org/10.1603/EC10040>.
- Tabashnik, B. E.; Gould, F. Delaying corn rootworm resistance to Bt corn. *Journal of Economic Entomology*, v.105, n.3, p. 767-776, 2012. <https://doi.org/10.1603/EC12080>.
- Waldbauer, G. P. The consumption and utilization of food by insects. *Advances in Insect Physiology*, v.5, p.229-288, 1968. [https://doi.org/10.1016/S0065-2806\(08\)60230-1](https://doi.org/10.1016/S0065-2806(08)60230-1).
- Wang, D.; Wang, Z.; He, K.; Cong, B.; Bai, S.; Wen, L. Temporal and spatial expression of Cry1Ab toxin in transgenic Bt corn and its effects on Asian corn borer, *Ostrinia furnacalis* (Guenée). *Zhongguo Nongye Kexue*, v. 37, n. 8, p. 1155-1159, 2004. <https://europepmc.org/abstract/cba/465373>. 07 Abr. 2017.
- Yang, F.; Kerns, D. L.; Brown, S.; Kurtz, R.; Dennehy, T.; Braxton, B.; Head, G.; Huang, F. Performance and cross-crop resistance of Cry1F-maize selected *Spodoptera frugiperda* on transgenic Bt cotton: implications for resistance management. *Scientific Reports*, v.6, e28059, 2016. <https://doi.org/10.1038/srep28059>.
- Yinghua, S.; Yan, D.; Jin, C.; Jiayi, W.; Jianwu, W. Responses of the cutworm *Spodoptera litura* (Lepidoptera: Noctuidae) to two Bt corn hybrids expressing Cry1Ab. *Scientific Reports*, v.7, e.41577, 2017. <https://doi.org/10.1038/srep41577>.