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Biological Control and Crop Protection

Larval development of *Spodoptera eridania* (Cramer) fed on leaves of *Bt* maize expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 proteins and its non-*Bt* isoline

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ABSTRACT

This study aimed to evaluate, in controlled laboratory conditions (temperature of 25±2 °C, relative humidity of 60±10%, and 14/10 h L/D photoperiod), the larval development of *Spodoptera eridania* (Cramer, 1784) (Lepidoptera, Noctuidae) fed with leaves of *Bt* maize expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 insecticide proteins and its non-*Bt* isoline. Maize leaves triggered 100% of mortality on *S. eridania* larvae independently of being *Bt* or non-*Bt* plants. However, it was observed that in overall *Bt* maize (expressing a single or pyramided protein) slightly affects the larval development of *S. eridania*, even under reduced leaf consumption. Therefore, these results showed that Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 can affect the larval development of *S. eridania*, although it is not a target pest of this plant; however, more research is needed to better understand this evidence. Finally, this study confirms that non-*Bt* maize leaves are unsuitable food source to *S. eridania* larvae, suggesting that they are not a potential pest in maize fields.

Introduction

Among the biological pest control technologies currently available in the market, the cropping of genetically modified plants, mainly expressing genes of *Bacillus thuringiensis* Berliner (*Bt*), has grown exponentially worldwide (James, 2013). The increasing use of OGMs is due to the reduced use of insecticides that this technology provides for pest management programs, consequently reducing costs for producers (Qaim and Mathuschke, 2005; Werle et al., 2011). However, this change of pest control strategy may favor the incidence of other non-target insects that are currently of secondary importance to non-*Bt* crops. Some changes in the population dynamics of insect-pests were already reported in different countries of Europe and South America, where *Bt*-maize favored the population growth of non-target pests such as aphids and leafhoppers, although the reason for these changes is not still very clear (Faria et al., 2007; Virla et al., 2010).

The non-target pests are those insects that through ingestion of *Bt*-plants are exposed to Cry toxic proteins for a long time period, but are not direct targets of such technology (Andow and Hilbeck, 2004). The farming areas grown with *Bt*-plants may favor the population increase of non-target pests for two main reasons: (1) reduction on use of broad spectrum insecticides, which increases mortality of the secondary pests (Lu et al., 2010), and (2) lower competition for food, what consequently may cause an increase in population of these non-target pests within the cropped area (Zeilinger et al., 2011). The caterpillar *Spodoptera eridania* (Cramer, 1784) (Lepidoptera, Noctuidae) is one of the lepidopteran species of secondary importance that are reported to occur in areas cultivated with maize (Manuwoto and Scriber, 1985). Such insect species is highly polyphagous and its host range includes horticultural plants (Michereff-Filho et al., 2008), fruiting plants (Bortoli et al., 2012; Zenker et al., 2010), and ornamental plants (Delaney, 2012). Its economic importance seems to be increasing in major annual crops, such as soybean and cotton (Bueno et al., 2011; Santos et al., 2005; Santos et al., 2010).

In face of the foregoing, it may be inferred that larvae of *S. eridania* display a wide capacity of adaptability to different agroecosys-

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tems, for they may use the leaves of various crops as food source. However, information regarding the impact of *Bt* technology on the development of high infestations of this pest on non-*Bt* maize crops is still scarce. Nevertheless, this information is crucial to the development of adequate planning of integrated pest management for both types of maize, *Bt* genotypes (Cry1F and Cry1F + Cry1A.105 + Cry2Ab2) as well as for its non-*Bt* isolines. Therefore, this study aimed at studying the comparative biology of *S. eridania* fed with leaves originating from two *Bt* maize isolines, expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 proteins, and its near non-*Bt* maize isoline.

Material and methods

Experimental conditions and insect colony

The study was carried out in the entomology laboratory of Embrapa Soybean, and the experiments were performed into BOD type climatic chambers under controlled environmental conditions of temperature (25 ± 2 °C), relative humidity (60 ± 10%), and photoperiod [14/10 h (L/D)]. Larvae of *S. eridania* used in the study were obtained in the insect mass rearing of Embrapa Soybean, where these insects have been reared for around 20 generations, following the methodology described by Pomari et al. (2012).

Maize plants

The two *Bt* maize isolines evaluated in the study were Herculex® I (Dow AgroSciences) and PowerCore® (Dow AgroSciences and Monsanto), and its near non-*Bt* isoline, the hybrid 2B688 DOW (Dow AgroSciences). Genotype Herculex® I (event TC1507) contains the gene encoding for the insecticidal protein Cry1F, and genotype PowerCore® (event MON89034 x MON00603 x DAS01507) contains five genes with stacking traits that encode for three different *Bt*-proteins (Cry1F + Cry1A.105 + Cry2Ab2) with insecticidal effect, and two genes (PAT + EPSPS) that encode for tolerance to the herbicides glyphosate and glufosinate (Santos et al., 2012).

Sowing of the three maize genotypes was performed into pots, each containing 8 L of sterilized soil as substrate, which were then transferred to a greenhouse. For all genotypes assessed (*Bt* and non-*Bt*) five seeds per pot were used, thereby totaling 75 plants to each genotype. Substrate irrigation was performed before sowing, as well as immediately after sowing, to ensure uniformity of soil moisture within all pots; after seedling emergence, irrigation was performed daily. Chemical fertilization was carried out in post emergence through the application of nitrogen [$(NH_4)_2SO_4$], phosphorus (P_2O_5) and potassium (K_2O), following the technical recommendations indicated to the crop (Fancelli and Dourado-Neto, 2000). Throughout the crop cycle application of herbicides, fungicides or insecticides was not carried out in order to avoid interference of those chemical products on the results obtained.

To feed the larvae, leaves from the three maize genotypes were collected daily, starting when plants reached the vegetative growth stage V_8 (eight fully developed leaves). Soon after collection, leaves were cut into pieces (about 30 cm^2 each), and before being offered to the larvae the leaf sections were disinfected by immersion in a 5% sodium hypochlorite solution for 15 minutes. After such period, sections were removed from the solution and left to dry for 30 minutes, for solution evaporation.

Biological characteristics of S. eridania *larvae when fed on Bt and non-Bt maize at the first and the third instar*

Two independent bioassays were carried out. In the first bioassay, newly hatched larvae (larvae up to 24 hours old) were individualized with the aid of a thin tip brush (0.6 mm) into paraffined cups with a 50 mL capacity, and fed according to the previously described treatments. Differently for the third-instar bioassay, the newly hatched larvae were fed an artificial diet (Kasten et al., 1978) until reaching the third instar, when they were then transferred to the respective treatments.

In both bioassays, larvae of each replicate were fed leaf sections previously prepared (treatment) and offered *ad libitum*. A cotton pad soaked in sterile water was placed at the base of each leaf section to slow the drying of leaves. Leaf sections were replaced daily at the same time in which the larval instar and the mortality rate and development (days) of larvae were daily assessed. The two bioassays were carried out in a completely randomized, experimental design, with three treatments [two *Bt* isolines (expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2) and its near non-*Bt* isoline (genotype 2B688 DOW)], and 10 replications. Each replicate was composed of eight larvae individualized into paraffined cups, thereby totaling 80 larvae to each treatment.

Biological characteristics and foliar consumption of fifth instar S. eridania *larvae fed Bt and non-Bt maize*

This bioassay was carried out following the same methodology previously described for both the first and the third instars bioassays. However, since these previous bioassays resulted in a 100% *S. eridania* mortality, in this essay the larvae were reared on an artificial diet until reaching the fifth instar, when they were then transferred to paraffined cups, and *Bt* and non-*Bt* maize leaves were offered *ad libitum* to larvae, and leaf consumption by the *S. eridania* larvae was assessed.

According to Bueno et al. (2011) over 90% of larvae consumption occurs at the 5th and the 6th instar, which indicates that this methodology is valid to compare this parameter between treatments. To assess foliar area reduction caused by the dehydration, the foliar sections of the maize leaves that remained in the paraffined cups without larva were measured and used as control, thereby allowing correction of the total leaf area consumed by the larvae. Assessments of leaf area consumed by the larvae were performed daily with the aid of a foliar area meter (brand LI-Cor, model LI-Cor AM 300), until the interruption of their feeding habits.

Statistical analysis

Results of the different bioassays were subjected to exploratory analyzes to assess the assumptions of normality of residuals (Shapiro and Wilk, 1965), the homogeneity of variance of treatments, and additivity of model (Burr and Foster, 1972), to allow for ANOVA application. The means were compared by Tukey test (SAS Institute Inc., 2001), and difference was considered significant only when the significance level was $P \leq 0.05$.

Results

Biological characteristics of S. eridania *larvae when fed on Bt and non-Bt maize at the first and the third instar*

Newly hatched *S. eridania* larvae (first instar) fed on leaves of both *Bt* isolines (expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 proteins) showed a slightly shorter development period (1 day) than larvae fed on leaves of the non-*Bt* genotype (1.7 days) (Table 1). Nevertheless, *Bt* and non-*Bt* maize leaves triggered 100% mortality during the first instar on *S. eridania* larvae (Table 2).

The second bioassay again showed a slight impact of *Bt* plants, and a higher mortality before larvae molting until fourth instar on *Bt* maize expressing Cry1F + Cry1A.105 + Cry2Ab2 proteins (Table 2). Among the surviving larvae that reached the fourth instar, the mortality rate was lower on the non-*Bt* maize, but it reached 73% (Table 2).

Table 1.
Larval development, consumption and mortality of *Spodoptera eridania* fed with a genotype non-*Bt* and two genetically modified maize isolines.

Diet provided	Larval biological aspects of *S. eridania**		
Maize leaves	Development period (days)		
	First instar	Third instar	Forth instar
Genotype non-*Bt* (DOW 2B688)	1.7 ± 0.13 a	3.69 ± 0.15 b	5.46 ± 0.21 a
Cry1F	1.0 ± 0.04 b	4.83 ± 0.25 a	2.97 ± 0.13 b
Cry1F + Cry1A.105 + Cry2Ab2	1.0 ± 0.06 b	4.92 ± 0.08 a	1.00 ± 0.24 c
CV (%)	19.48	12.5	54.57
$DF_{residual}$	27	27	27
F	21.0	16.14	17.16
p	< 0.01	< 0.01	< 0.01
Diet provided	Larval biological aspects of *S. eridania**		
Maize leaves	Development period (days)		Leaf consumption (cm^2)
	Fifth instar	Sixth instar	Fifth and Sixth instars
Genotype non-*Bt* (DOW 2B688)	8.50 ± 0.28 b	10.70 ± 0.79 a	58.88 ± 4.85 a
Cry1F	8.68 ± 0.15 b	9.52 ± 0.29 a	37.55 ± 1.81 b
Cry1F + Cry1A.105 + Cry2Ab2	10.40 ± 0.53 a	4.80 ± 1.06 b	42.19 ± 1.48 b
CV (%)	9.48	53.32	6.20
$DF_{residual}$	12	12	12
F	7.23	7.46	6.82
p	< 0.01	0.01	< 0.01

* Means ± SEM followed by the same letter in the columns at the same larval instar do not statistically differ between each other, by Tukey test ($p \leq 0.05$).

Table 2.
Mortality (%) of *Spodoptera eridania* fed with a genotype non-*Bt* and two genetically modified maize isolines.

Diet provided	Larval biological aspects of *S. eridania*		
Maize leaves	Mortality (%)		
	First instar	Third instar	Forth instar*
Genotype non-*Bt* (DOW 2B688)	100.00 ± 0.00^{ns}	41.00 ± 2.45 b	73.00 ± 2.76 b
Cry1F	100.00 ± 0.00	73.00 ± 3.86 ab	100.00 ± 0.00 a
Cry1F + Cry1A.105 + Cry2Ab2	100.00 ± 0.00	95.00 ± 3.92 a	100.00 ± 0.00 a
CV (%)	0.00	22.7	12.5
$DF_{residual}$	27	27	27
F	0.00	5.14	6.16
p	0.05	< 0.05	< 0.05

Means ± SEM followed by the same letter in the columns at the same larval instar do not statistically differ between each other, by Tukey test ($p \leq 0.05$); ^{ns} = non significance; * = specimens that survived by third instar bioassay. All data were transformed in arcsen $\sqrt{X+1}$.

Third instar larvae took a shorter time to complete this instar (3.69 days) compared to the larvae fed on *Bt* maize leaves (4.83-4.92 days) (Table 1). The larvae that molted to fourth instar took 5.46 days more to molt until the next instar (fifth) when feeding on non-*Bt* maize leaves, while no larvae completed the fourth instar when feeding on *Bt* maize, taking 2.97 and 1 days to die on Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 treatments, respectively (Table 1).

Biological characteristics and foliar consumption of fifth instar S. eridania *larvae fed with Bt and non-Bt maize*

When leaf consumption of *S. eridania* larvae was assessed at the fifth and at the sixth larval instar, the impact of *Bt* cultivars Cry1F + Cry1A.105 + Cry2Ab2 and of that with only one insecticide protein Cry1F on the larvae feeding capacity was evident. While *S. eridania* leaf consumption was 58.88 cm^2 on non-*Bt* isoline, it only reached 37.55 and 42.19 cm^2 on *Bt* isolines expressing Cry1F and Cry1F + Cry1A.105 + Cry2Ab2, respectively (Table 1).

Furthermore, when the biological aspects of *S. eridania* larvae at the fifth and sixth instars were assessed (from larvae which were fed an artificial diet until the fifth instar and in treatments only offered at the fifth and the sixth instar), maize leaves still triggered 100% mortality, independently of being *Bt* or non-*Bt* plants, with all larvae being unable of undergoing the pupal stage (Table 2). At the sixth instar, *S. eridania* death occurred faster in *Bt* maize expressing Cry1F + Cry1A.105 + Cry2Ab2 proteins (development period of 4.8 days) compared to both other treatments, of which larvae development was around 10 days (Table 1). Despite the fact that *Bt* maize expressing Cry1F + Cry1A.105 + Cry2Ab2 proteins killed sixth instar *S. eridania* caterpillars faster (4.80 days) than *Bt* maize only expressing Cry1F (9.52 days), this did not result in a lower injury. Both *Bt* maize evaluated allowed similar *S. eridania* leaf consumption (Table 1).

Discussion

Spodoptera eridania larvae fed with leaves of both *Bt* maize genotypes (expressing both Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 proteins) showed a slightly shorter development period (days), although this was not enough to reduce leaf consumption. In South Africa, Van den Berg and Van Wyk (2007) studied the impact of the Cry1Ab protein, present in *Bt* maize genotypes, on the non-target larvae of the African pink stem borer (*Sesamia calamistis* Hampson, 1910) (Lepidoptera, Noctuidae) and observed a lower larval development of this pest when fed with leaves of the evaluated *Bt* maize genotype.

Such results indicate that the spectrum of action of *Bt* proteins may be broader than first expected, and this impact on non-target pests may aid to the pest management, since the population of insect-pests, even those considered of minor importance may be maintained at relatively low levels. In our study it was demonstrated that the two isolines of *Bt* maize, cv Herculex® I (event TC1507 – expressing Cry1F protein) and cv Powercore® (event MON89034 x MON00603 x DAS01507 – expressing Cry1F + Cry1A.105 + Cry2Ab2 proteins) impaired *S. eridania* development. However, as this study indicated that maize leaves are an inappropriate food, it is difficult to measure the potential of this *Bt* maize to regulate this pest insect. Since both *Bt* genotypes assessed had similar results regarding *S. eridania* management (lower development larval), it could be an indication of the lack of Cry1A.105 or Cry 2Ab2 benefits, considering only a possible *S. eridania* management use. However, it is suggested that to ensure the success of the Insect Resistance Management (IRM) and therefore reduce insect resistant selection, the use of *Bt* plants having more than one protein active against pest species is most of the time desirable (Storer et al., 2012).

It is important to point out though, that benefits of stacking different *Bt* proteins should not be generalized, since the result might be unexpected. This fact has already been evidenced by Bergamasso et al. (2012), who in a study in which the effect of the Cry1Ia10 protein in association with protein Vip3Aa has been assessed, have found that such effect was highly successful in controlling different larvae species of the *Spodoptera* complex; nevertheless, for the control of *S. eridania* the effect of these two proteins was not efficient.

Despite some reports of *S. eridania* larvae in non-*Bt* maize fields, currently, in this study it was shown that larvae of this species have a low biotic potential when feeding only on leaves of this crop. The fact that the *S. eridania* larvae cannot completely develop when fed with maize leaves might be explained by three hypothesis: (1) nutritional insufficiency (possibly due to the low adaptability of the larvae to leaves of this crop); (2) presence of some chemical compounds in maize leaves, which may have impaired the normal development of the larvae of this species, or (3) non preference. So, all possibilities are strongly related to the nutritional quality of maize plants. In this sense, Fraenkel (1959) had already reported that despite the similarity in nutritional value of most plants to phytophagous insects, the presence of many allelochemical substances determines significant differences in the use of the nutrients found in these plants by the insects. Therefore, adaptability of a given host may arise from the capacity of the insect to metabolize some of those components, whose nutritional function is not recognized by the insect (Schoonhoven and Meerman, 1978). As an example, *S. frugiperda* exhibited a preference of feeding on non-*Bt* maize, compared to *Bt* isolines expressing Cry1A (b) protein (Mendes et al., 2011).

Some studies conducted under laboratory environmental conditions have already shown that the tannin (a secondary metabolite in the maize leaf) and the zein (the major maize protein) are highly detrimental to development of larvae of *S. eridania*, as they may cause changes in some biological characteristics of these larvae, such as food conversion rate and larval growth rate due to the greater metabolic expenditure, and hence the lower weight of larvae (Karowe and Martin, 1989; Manuwoto et al., 1985). As the two compounds (tannin and zein) are present in the maize leaves (Azim et al., 1989; Bhaigyabati et al., 2011), it is possible that these compounds have affected the development of the *S. eridania* larvae assessed in this study.

According to Karowe and Martin (1989) ingestion of zein might cause unbalance on larvae metabolism by inducing large losses of nitrogen, due to the excessive production of uric acid, which may cause death of larvae. In this study similar results were found; however, it was also observed, that possibly due to problems in the metabolism, larvae have released their body fluids through the two extremities. These symptoms have always been most evident in the larvae of first and third instars, probably because they are more sensitive to ingestion of the protein during the first instars. However, although the mortality rate of the fifth instar larvae was relatively low when they were fed with leaves of the non-*Bt* genotype, even the larvae that reached the sixth instar have failed to reach pupal stage, thus proving that maize leaves do not have a suitable nutritional quality for the development of *S. eridania* larvae.

Nevertheless, the nutritional aspect of the *S. eridania* larvae deserves to be further studied. Moreover, it is necessary to consider that the occurrence of *S. eridania* larvae is still sporadic in areas cultivated with maize, and the knowledge on the bioecology of this larvae species is still very limited. In addition, our results indicated that Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 slightly affected the larval development of *S. eridania*, but more studies using these strains are needed to confirm this evidence.

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Conflicts of interest

The authors declare no conflicts of interest.

References

Andow, D.A., Hilbeck, A., 2004. Science-based risk assessment for non-target effects of transgenic crops. Bioscience 54, 637-649.

Azim, A., Nasser, Z., Ali, A., 1989. Nutritional evaluation of maize fodder at two different vegetative stages. Asian Australas. J. Anim. Sci. 2, 27-34.

Bergamasso, V.B., Mendes, D.R.P., Fernandes, O.A., Desidério, J.A., Lemos, M.V.F., 2012. *Bacillus thunringiensis* Cry1Ia10 and Vip3Aa protein interactions and theirs toxicity in *Spodoptera* spp. (Lepidoptera). J. Invertebr. Pathol. 112, 152-158.

Bhaigyabati, T., Kirithika, T., Ramya, J., Usha, K., 2011. Phytochemical constituients and antioxidant activity of various extracts of corn silk (*Zea mays* L.). Res. J. Phar. Biol. Chem. Sci. 2, 983-986.

Bortoli, L.C., Bertin, A., Efrom, C.F.S., Botton, M., 2012. Biologia e tabela de vida de fertilidade de *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae) em morangueiro e videira. Rev. Bras. Fruticultura 34, 1068-1073.

Bueno, R.C., Bueno, A.F., Moscardi. F., Parra, J.R., Hoffmann-Campo, C.B., 2011. Lepidopteran larva consumption of soybean foliage: basis for developing multiple-species economic thresholds for pest management decisions. Pest. Man. Sci. 77, 170-174.

Burr, I.W., Foster, L.A., 1972. A test for equality of variances. Mimeograph Series number 282. University of Purdue, West Lafayette.

Delaney, K.J., 2012. *Nerium oleander* indirect leaf photosynthesis and light harvesting reductions after clipping injury or *Spodoptera eridania* herbivory: high sensitivity to injury. Plant Sci. 185-186, 218-226.

Fancelli, A.L., Dourado-Neto, D., 2000. Produção de Milho. Ed. Agropecuária, Guaíba.

Faria, C.A., Wäckers, F.L., Pritchard, J., Barrett, D.A., Turlings, T.C.J., 2007. High susceptibility of Bt maize to aphids enhances the performance of parasitoids of lepidopteran pests. PLoS One 2, 1-11.

Fraenkel, G.S., 1959. The raison d'être of secondary plant substances. Science 129, 1466-1470.

James, C., 2013. ISAAA Brief N. 46. Global Status of Commercialized Biotech/GM Crops: 2013. Ithaca.

Karowe, D.N., Martin, M.M., 1989. The effects of quantity and quality of diet nitrogen on the growth, efficiency of food utilization, nitrogen budget, and metabolic rate of fifth-instar *Spodoptera eridania* larvae (Lepidoptera: Noctuidae). J. Insect Physiol. 35, 699-708.

Kasten Jr., P., Precetti, A.A.C.M., Parra, J.R.P., 1978. Dados biológicos comparativos de *Spodoptera frugiperda* (J.E. Smith, 1797) em duas dietas artificiais e substrato natural. Rev. Agric. 53, 69-78.

Lu, Y., Wu, K., Jiang, Y., Xia, B., Li, P., Feng, H., Wyckhuys, K.A.G., Guo, Y., 2010. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of *Bt* cotton in China. Science. 328, 1151-1154.

Manuwoto, S., Scriber, J. M. 1985. Differential effects of nitrogen fertilization of three corn genotypes on biomass and nitrogen utilization by the southern armyworm, Spodoptera eridania. Agric. Ecos. Environ. 14, 25-40.

Mendes, S.M., Boregas, K.G.B., Lopes, M.E., Waquil, M.S., Waquil, J.M., 2011. Respostas da lagarta-do-cartucho a milho geneticamente modificado expressando a toxina Cry1A(b). Pesq. Agropec. Bras. 46, 239-244.

Michereff-Filho, M., Torres, J.B., Andrade, L.N.T., Nunes, M.U.C., 2008. Effect of some biorational insecticides on *Spodoptera eridania* in organic cabbage. Pest. Man. Sci. 64, 761-767.

Pomari, A.F., Bueno, A.F, Bueno, R.C.O.F., Menezes Jr., A.O.M., 2012. Biological characteristics and thermal requirements of the biological control agent *Telenomus remus* (Hymenoptera: Platygastridae) reared on eggs of different species of the genus *Spodoptera* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 105, 73-81.

Qaim, M., Matuschke, I., 2005. Impacts of genetically modified crops in developing countries: A survey. Quar. J. Int. Agric. 44, 207-217.

Santos, K.B., Meneguim, A.M., Neves, P.M.O.J., 2005. Biologia de *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae) em diferentes hospedeiros. Neotrop. Ent. 34, 903-910.

Santos, A.C., Rosseto, J, Marques, L.H.S.F., 2012. Geração Bt. Caderno Técnico Cultura do Milho. Cultivar Grandes Culturas.

Santos, K.B., Meneguim, A.M., Santos, W.J., Neves, P.M.O.J., Santos, R.B., 2010. Caracterização dos danos de *Spodoptera eridania* (Cramer) e *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae) a estruturas de algodoeiro. Neotrop. Ent. 39, 626-631.

SAS Institute Inc., 2001. SAS/STAT® 8.2 User's Guide. Cary, SAS Institute Inc.

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika. 52, 591-611.

Schoonhoven, L.M., Meerman, J., 1978. Metabolic cost of changes in diet and neutralization of allelochemicals. Ent. Exper. Appl. 24, 689-693.

Storer, N.P., Kubiszak, M.E., Ed King, J., Thompson, G.D., Santos, A.C., 2012. Status of resistance to Bt maize in *Spodoptera frugiperda*: Lessons from Puerto Rico. J. Invertebr. Pathol. 110, 294-300.

Van den Berg, J., Van Wyk, A., 2007. The effect of Bt maize on *Sesamia calamistis* in South Africa. Ent. Exper. Appl. 122, 45-51.

Virla, E.G., Casuso, M., Frias, E.A., 2010. A preliminary study on the effects of a transgenic corn event on the non-target pest *Dalbulus maidis* (Hemiptera: Cicadellidae). Crop Protect. 29, 635-638.

Werle, A.J.K., Nicolay, R.J., Santos, R.F., Borsoi, A., Secco, D., 2011. Avaliação de híbridos de milho convencional e transgênico (Bt), com diferentes aplicações de inseticida em cultivo safrinha. Ver. Bras. Tec. Apl. Ciênc. Agr. 4, 150-168.

Zeilinger, A.R., Olson, D.M., Andow, D.A., 2011. Competition between sting bug and heliothine caterpillar pests on cotton at within-plant spatial scales. Ent. Exper. Appl. 141, 59-70.

Zenker, M. M., Botton, M., Teston, J. A., Specht, A., 2010. Noctuidae moths occurring in grape orchards in Serra Gaúcha, Brazil and their relation to fruit-piercing. Rev. Bras. Entomol. 54, 288-297.