

Yield Losses in Transgenic Cry1Ab and Non-Bt Corn as Assessed Using a Crop-Life-Table Approach

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Abstract

In this study, we constructed crop life tables for *Bacillus thuringiensis* Berliner (Bt) Cry1Ab and non-Bt corn hybrids, in which yield-loss factors and abundance of predaceous arthropods were recorded during 2 yr at two locations. Corn kernel/grain was the yield component that had the heaviest losses and that determined the overall yield loss in the corn hybrids across years and locations. Yield losses in both corn hybrids were primarily caused by kernel-destroying insects. *Helicoverpa zea* (Boddie) and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) were the key loss factors at one location, while at the other, the key loss factor was the silk fly larvae, *Euxesta* spp. (Diptera: Ulidiidae). Although the realized yield of corn grains was not different ($P > 0.05$) between Cry1Ab and non-Bt corn hybrids, the Bt corn hybrid reduced ($P < 0.05$) the damage by *H. zea* and *S. frugiperda* in three of the four field trials, particularly at the location where Lepidoptera were the key loss factors. As expected, no reduction in the abundance of predaceous arthropods was observed in Cry1Ab corn fields. Various species of natural enemies were recorded, particularly the earwig *Doru luteipes* (Scudder) (Dermaptera: Forficulidae), which was the most abundant and frequent predaceous insect. These results indicate that integration of pest management practices should be pursued to effectively minimize losses by kernel-destroying insects during corn reproductive stages when growing non-Bt or certain low-dose Bt corn cultivars for fall armyworm and corn earworm, such as those producing Cry1Ab or other Cry toxins.

Key words: maize, Bt toxin, corn earworm, fall armyworm, silk fly, natural enemy

Transgenic crops expressing insecticidal toxins from the soil bacterium *Bacillus thuringiensis* Berliner (Bt) have been adopted in insect pest management programs worldwide (Sanahuja et al. 2011, ISAAA 2016). Transgenic Bt corn expressing the Cry1Ab toxin (e.g., MON810, Bt11) was commercially introduced in several countries of the Americas, Asia, and Africa (CERA 2017) targeting important Lepidoptera pests, such as the sugarcane borer *Diatraea saccharalis* (Fabricius) (Crambidae), corn earworm *Helicoverpa zea* (Boddie) (Noctuidae), European corn borer *Ostrinia nubilalis* (Hübner) (Crambidae), and fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Noctuidae) (Chaufaux et al. 2001, Binning and Rice 2002, Buntin 2008). Some studies, however, has reported low or reduced efficacy of Bt Cry1Ab corn against some target insects due to resistance development or its less-than-high-dose status for some key-noctuid pests (Ghimire et al. 2011, Omoto et al. 2016, Sousa et al. 2016).

A number of approaches have been proposed to document yield-loss factors in crop production (Harcourt 1970, Picanço 1992, Oerke 2006, Savary et al. 2006). The crop life table tool, which is based on methods and concepts pioneered by Varley and Gradwell (1960) and latter adapted by Picanço (1992), can be used to quantify yield-loss factors in different crop stages (Harcourt 1970; Picanço et al. 1998, 2007; Bacci et al. 2006; Pereira et al. 2017). By using this analytic tool, one can determine the critical yield component and the key loss factor, which can be used to inform decisions on crop management. The critical yield component is that which either explains the largest proportion of variation of the total crop losses or alternatively the component that undergoes the highest losses. The key loss factor is either that showing the strongest correlation with losses in the critical yield component or, alternatively, the factor that leads to the heaviest losses in the critical component of yield (Bacci et al. 2006). Thus, using this life-table approach the primary components

of yield loss can be identified for both Bt and non-Bt corn hybrids, and changes in the status of non-target secondary pests in Bt corn fields can be evaluated, with the ultimate goal of developing a more suitable plan of integrated pest management in corn.

Despite the importance of determining crop yield losses to inform strategic management decisions, few studies have tried to assess, quantify, or compare the yield-limiting factors in the yield components of Bt crops (Head et al. 2005, Hutchison et al. 2010). Also, some studies have assessed the effect of Bt cultivars on the natural enemy populations (Naranjo 2005, Lu et al. 2012), but not much data are available for tropical and subtropical regions, where the arthropod fauna is rich and abundant. Here we determined the critical yield component and key loss factors in transgenic Cry1Ab corn and non-Bt corn using a crop-life-table approach. In addition, we determined the abundance of natural enemies in each corn hybrid. The present research has implications for documenting primary yield loss components in corn crops, assisting pest management decisions, and assessing the impact of a Bt corn hybrid on the abundance of predaceous insects in the field.

Materials and Methods

Locations, Experimental Design, and Crop Management Practices

This study was conducted for 2 yr in two different areas (Location 1: 20°47'27"S, 42°47'49"W, altitude 679 m, with no history of growing annual crops; and Location 2: 20°45'37"S, 42°52'04"W, altitude 648 m, with a history of growing annual crops), in Viçosa, Minas Gerais State, Brazil. Corn was grown for two consecutive years, in 2009–2010 (from 22 October 2009 to 23 February 2010, hereinafter named year 1) and again in 2010–2011 (from 3 November 2010 to 4 March 2011, hereinafter named year 2). This represents the primary season that corn is grown in Brazil despite having a second crop in some regions of the country.

The Bt Cry1Ab corn hybrid (DKB 390 YG, event MON 810, Monsanto do Brasil, São Paulo, SP) and its non-transgenic isohybrid (DKB 390, Monsanto do Brasil, São Paulo, SP) were used in this study. Limestone and fertilizers (i.e., macro- and micronutrients) were applied at same rates for Bt and non-Bt corn plots, following technical recommendations based on soil analysis at each location. No pesticides (to control insects or plant diseases) were applied. The experimental design was completely randomized with eight replicates at location 1 and seven replicates at location 2. Each replicate consisted of a 200 m² area containing 14 rows with 95 plants in each row. The plants were spaced 0.2 m within a row and 0.75 m between rows, and the overall plant density was 66,667 plants ha⁻¹.

Assessing Yield and Yield-Loss Factors

Corn plant mortality was monitored daily from the vegetative stage to the reproductive stage using methods adapted from Picanço et al. (2003) and Bacci et al. (2006). The corn vegetative stage comprised the period from germination to tasseling. During this stage, daily assessments were performed to identify the insect species that attacked the corn plants, particularly the roots, seeds, and seedlings (young plants). These species included *Dichelops furcatus* (Fabricius) (Hemiptera: Pentatomidae), termites, *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), and *Gryllus* spp. (Orthoptera: Gryllidae). Possible attacks by the fall armyworm, aphids, and leafhoppers were assessed during the entire corn development. Plant mortality was attributed to a particular insect species when it was observed feeding on the plant tissue or based on the

attack symptoms typical of each pest species. To confirm the identification of insects that were recorded as loss factors, a few specimens of each morphospecies were collected, placed in 70% ethanol, and brought to the laboratory, where they were examined using taxonomic keys (Triplehorn et al. 2005) and compared with specimens in the Regional Museum of the Federal University of Viçosa. Plant mortality was attributed to fungi when dead plants were found (dry or permanently wilted) with disease symptoms (CIMMYT 2004). These plants were collected, placed in individual plastic bags, and sent to the phytopathology laboratory at the Plant Disease Clinic of the Federal University of Viçosa to confirm the causal pathogen.

The corn reproductive stage was defined as being from the beginning of the silking period to physiological maturation of kernels. During this stage, daily evaluations were performed to identify the loss factors and record the number of corn ears per plant. Plants that did not produce corn ears or had some of them aborted were also recorded and labeled in the field. The loss factor recorded for kernels was abortion. In each plot, 10 corn ears were randomly collected, placed in paper bags, and examined to identify the loss factors using a stereoscopic microscope when needed (Motic K-400L; Ted Pella, Inc., Redding, CA). The number of grains in the corn ears was recorded as was the number of intact grains and those damaged by each of the loss factors as follows. Germinated grains were identified by the presence of the sprout on the grain surface. Malformed grains were identified by comparison with healthy grains and with those of different shapes found on the same corn ear. Kernel damage was attributed to insects when individuals of a particular insect species were observed feeding on the ear or when symptoms or signs typical of its attack were identified. Several insect species were recorded attacking corn ears and causing heavy kernel losses, including *H. zea*, *S. frugiperda*, *Euxesta* spp. (Diptera: Ulidiidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Cathartus quadricollis* Guérin-Ménéville (Coleoptera: Silvanidae), and *Carpophilus* sp. (Coleoptera: Nitidulidae). We distinguished *H. zea* and *S. frugiperda* kernel feeding based on the portion of the ear that is typically colonized by the larvae of each species. Whereas *H. zea* larvae enter the husk and feed on the kernels near the ear tip, *S. frugiperda* bore into the husks from the lower part of the ear, feeding on corn kernels from the lower part of the ear. Kernels infected by fungi were identified by the fungal spores on the grain surfaces and other symptoms (CIMMYT 2004). Finally, rat attack was identified when part of the corncob, husks, and kernels were destroyed by jaws of a small rodent, which is typical of rat feeding.

The realized corn yield was estimated when the plants were ready for harvest. In each plot, 100 plants were randomly selected, and their ears were harvested. The grains were removed from the corn ears, counted, and dried at 60°C in a forced-air oven (Marconi MA 037/5, Campinas, São Paulo, Brazil) until constant mass. Grain mass was determined with a digital balance (OHAUS AV8101P, Ohaus Corporation, Parsippany, NJ) and converted to grain weight at 12% moisture content.

Estimating Losses Within Each Yield Component

In our modified life-table approach (Picanço 1992; Picanço et al. 1998, 2003), the corn yield components were plants, ears, and grains, in which we estimated yield losses. Corn grain productivity (i.e., yield) and yield losses in each yield component were estimated in each experimental replicate. The potential yield (i.e., the yield at emergence) was estimated using the following equation (Picanço et al. 2003):

$$Y_{pot} = NPA \times NEP \times NGE \times WG \times F,$$

where Y_{pot} is the potential corn yield (kg ha⁻¹); NPA is the number of plants alive per plot (200 m²) at the emergence; NEP is the mean number of corn ears per plant (estimated in 100 plants); NGE is the mean number of grains per ear; WG is the mean weight of one corn grain (kg), and F (set to 50) is a factor to convert the yield in each replication (i.e., 200 m²) to the yield in 1 ha (i.e., 10,000 m²).

In the vegetative stage, the yield loss from each factor (due to plant mortality) was estimated using the following equations:

$$Y_{LPM} = \sum y_{lpmi};$$

$$y_{lpmi} = npki \times NEP \times NGE \times WG \times F,$$

where Y_{LPM} is the estimated yield loss (kg ha⁻¹) from plant mortality; $\sum y_{lpmi}$ is the sum of estimated losses (kg ha⁻¹) from each mortality factor i in the vegetative stage, and $npki$ is the number of plants killed by each factor i in the vegetative stage ($i = E. lignosellus, S. frugiperda$, termites, *Gryllus* spp., *D. furcatus*). The other terms in the equation (NEP, NGE, WG, F) are those described previously.

Likewise, the yield loss from ears (during the reproductive stage) was estimated using the following equation:

$$Y_{LPE} = NPWE \times NEP \times NGE \times WG \times F,$$

where, Y_{LPE} is the estimated yield loss from plants without ears (kg ha⁻¹), and $NPWE$ is the number of plants that did not form ear. In this stage, grain losses were estimated in each replication using following equations (Picanço 1992; Picanço et al. 1998, 2003):

$$Y_{LG} = \sum y_{lgi}$$

$$y_{lgi} = nglj \times NEP \times NGE \times WG \times F,$$

where Y_{LG} is the estimated yield loss (kg ha⁻¹) from grain losses; y_{lgi} is the estimated loss (kg ha⁻¹) from factor j ; and $nglj$ is the number of grains lost from factor j in the reproductive stage ($j =$ each factor causing grain loss, listed in Table 1).

Finally, the realized crop yield was calculated using the following equation:

$$Y_{real} = Y_{pot} - (Y_{LPM} + Y_{LPE} + Y_{LG}),$$

where Y_{real} is the realized yield, Y_{pot} is the potential yield, and Y_{LPM}, Y_{LPE} , and Y_{LG} are yield losses (kg ha⁻¹) from plant mortality, plants without ear, and grain damage or destruction.

Assessing Population Levels of Natural Enemies

The densities of natural enemies were also monitored during the crop development in parallel with the study described above. In each plot, 10 plants were randomly selected and used to record the predaceous arthropods observed (only visually, not touching the plants). Next, the under and upper sides of the corn leaves were examined by carefully turning each leaf to prevent the arthropods from falling off or escaping. This assessment was performed once a week during the entire corn reproductive stage, and the specimens collected from the experimental plots were stored in glass bottles (10 ml) containing 70% ethanol and identified as described previously for the pest species.

Statistical Analysis

Before analyses, we checked whether the data were normally distributed and had homogenous variance using residual analyses of

Table 1. Yield losses (mean ± SE, kg ha⁻¹) in a transgenic Bt Cry1Ab and non-Bt corn hybrids, recorded for 2 yr at two locations

Corn yield component	Year 1		Year 2		Overall mean		
	Location 1		Location 2				
	Cry1Ab	Non-Bt	Cry1Ab	Non-Bt			
Plants/loss factor	0.0 ± 0.0	0.0 ± 0.0	87.2 ± 11.5	63.6 ± 9.0	14.1 ± 14.1	4.3 ± 4.3	30.4 ± 10.7
<i>S. frugiperda</i>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 1.6*	17.2 ± 5.0	2.3 ± 1.0
Termites	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.7 ± 5.1	29.6 ± 16.0	4.5 ± 2.5
<i>D. furcatus</i>	0.0 ± 0.0	0.0 ± 0.0	87.2 ± 11.5	63.6 ± 9.0	20.1 ± 14.6	0.00 ± 0.00	23.6 ± 5.1
Ears	176.1 ± 16.3	151.0 ± 15.1	16.1 ± 4.3	13.6 ± 4.6	428.2 ± 55.5	424.9 ± 51.6	170.0 ± 23.5
Grains/loss factor	1081.4 ± 311.4	1412.2 ± 74.6	1763.9 ± 258.0	1787.9 ± 240.6	298.50 ± 633.9	3729.6 ± 376.7	1879.5 ± 197.4
<i>C. quadricolis</i>	21.7 ± 9.51	37.7 ± 20.1	34.7 ± 22.7	0.0 ± 0.0	57.9 ± 16.9	96.8 ± 21.9	31.3 ± 11.9
<i>Euxestia</i> spp.	63.12 ± 41.2	41.1 ± 20.78	780.9 ± 211.4	1070.3 ± 244.9	0.0 ± 0.0	0.0 ± 0.0	275.3 ± 145.7
<i>H. zea</i>	336.3 ± 106.1*	806.2 ± 117.5	15.1 ± 15.1	0.0 ± 0.0	171.5.5 ± 506.1*	2818.0 ± 962.0	718.4 ± 366.4
<i>S. frugiperda</i>	0.0 ± 0.0*	41.2 ± 39.2	0.0 ± 0.0	0.0 ± 0.0	175.2 ± 70.9*	326.7 ± 64.7	76.2 ± 41.5
<i>S. zeamais</i>	24.2 ± 18.6	109.9 ± 81.6	27.0 ± 13.3	37.6 ± 9.1	48.6 ± 44.9	110.1 ± 89.7	75.4 ± 17.1
Fungi	3.0 ± 2.8	2.4 ± 1.9	3.0 ± 1.9	1.5 ± 1.5	16.8 ± 16.5	146.4 ± 53.4	26.6 ± 17.5
Germination	0.0 ± 0.0	0.0 ± 0.0	67.9 ± 34.9	25.5 ± 16.7	70.1 ± 40.4	18.1 ± 4.3	35.1 ± 11.7
Malformation	633.1 ± 202.2	373.7 ± 103.9	835.3 ± 18.7	653.0 ± 8.0	900.9 ± 219.6	213.5 ± 128.3	641.1 ± 84.9

*Significant difference ($P < 0.05$, ANOVA) between Cry1Ab and non-Bt corn within location and year. *E. lignosellus* and *Gryllus* spp., caused some plant death while *Carpophilus* sp. and rats caused a low grain loss; these factors were not listed because of lack of differences and low yield loss (3 kg ha⁻¹).

the particular linear model (PROC MIXED, PROC UNIVARIATE, PROC GPLOT, Littell et al. 2007, SAS Institute 2013), and no transformation was needed to meet the linear model or analysis of variance (ANOVA) assumptions. The data on corn yield and yield losses in each year were analyzed by multifactor ANOVA (two corn genotypes within two locations; all considered fixed factors) using the PROC MIXED procedure in SAS (Littell et al. 2007, SAS Institute 2013) with $\alpha = 0.05$. Yield losses caused by Lepidoptera larvae (i.e., *H. zea*, *S. frugiperda*), were compared between the corn hybrids within year and location using a one-way ANOVA ($\alpha = 0.05$).

To determine the critical yield component and key loss factor, using a k-factor analysis, the yield-loss data were transformed to log₁₀ in each experimental replication, obtaining the total loss (*K*) and the partial loss from a factor (*ki*) (Varley and Gradwell 1960, Bacci et al. 2006, Pereira et al. 2017). The total *K* loss was obtained by the sum of the *k* losses, (i.e., $K = \sum ki$), where $ki = \log(YLi)$, where *YL*i** is the loss occurred in each yield component (i.e., *i* = plant, ear, and grain). Next, simple linear regression analysis of each *k* partial loss on the *K* loss was performed (PROC REG, SAS Institute 2013). The partial loss component that showed the steepest slope (different from the others, *t*-test, $P \leq 0.05$) was recognized as the critical component of yield loss. A similar procedure was used when determining the key loss factor within the critical yield component (Picanço et al. 2007, Pereira et al. 2017). Using a one-way ANOVA ($\alpha = 0.05$), we compared the yield losses caused by the key factors in Cry1Ab and non-Bt corn hybrids at each location.

In addition, we calculated the mean population density of natural enemies per experimental plot during the corn reproductive stage for each year at both locations. To test whether such population density was similar between corn hybrids (Cry1Ab and non-Bt), data of location and year were combined and analyzed using ANOVA (PROC MIXED, PROC UNIVARIATE, PROC GPLOT, Littell et al. 2007, SAS Institute 2013). Year and its interactions were considered random factors while corn hybrid and location were fixed factors.

Results

The Bt Cry1Ab and non-Bt corn hybrids had potential and realized yields (Fig. 1A and C) and yield loss (Fig. 1B) that were not significantly different ($P > 0.05$) regardless of the location and the year. In contrast, the corn yield was larger at location 2 (with historical agricultural land use) than at location 1 (which had no history of growing annual crops) (Fig. 1A and C). Likewise, corn yield losses at both locations were not significantly different in year 1, but in year 2, the losses in corn yield at location 1 were larger than those at location 2 (Fig. 1).

The mean loss in each yield component and the loss caused by each factor within a given yield component is shown in Table 1. Averaged across year, location and corn hybrid, yield losses were greatest in the grains (1879.5 kg ha⁻¹, Table 1: overall mean column; see also Fig. 3A for mean loss values for each treatment combination). Conversely, the yield loss caused by plant mortality (i.e., in the plant yield component) was least (30.4 kg ha⁻¹) while the yield loss in ears was intermediate (170.0 kg ha⁻¹) (Table 1, Fig. 3A). Plant mortality were caused mainly by the stink bug *D. furcatus* (overall mean loss \pm SE, 23.6 \pm 5.1 kg ha⁻¹, Table 1), followed by termites (Isoptera: Termitidae), *E. lignosellus*, *Gryllus* spp., and *S. frugiperda* (Table 1). The only factor causing loss of ears was abortion (overall mean loss \pm SE, 170.0 \pm 23.5 kg ha⁻¹). In the grain yield component, losses were caused by the following factors (in descending magnitude): insects (overall mean loss \pm SE, 1177 \pm 369 kg ha⁻¹), poor grain formation, germination of grains in the ear, fungi, and rats

(Table 1). Insect species that caused grain losses were *H. zea*, *Euxesta* spp., *S. frugiperda*, *S. zeamais*, *C. quadricollis*, and *Carpophilus* sp. (Table 1). For each year of the experiment, the estimated yield losses caused by these insects across the locations were as follows (mean loss \pm SE): *H. zea* (year 1: 289 \pm 70, year 2: 1148 \pm 270 kg ha⁻¹); *Euxesta* spp. (year 1: 489 \pm 117, year 2: 62 \pm 26 kg ha⁻¹); *S. frugiperda* (year 1: 10 \pm 9, year 2: 142 \pm 34 kg ha⁻¹); *S. zeamais* (year 1: 50 \pm 20, year 2: 101 \pm 36 kg ha⁻¹); and *C. quadricollis* (year 1: 24 \pm 8, year 2: 39 \pm 10 kg ha⁻¹). Interestingly, the Bt Cry1Ab corn did not reduce the grain yield losses by Lepidoptera (*H. zea* and *S. frugiperda*) only in the first year and at location 1 (Table 1).

In the analyses to determine the critical yield component of corn, the highest proportional yield losses occurred in the ear component in all locations, corn hybrids, and years (Fig. 2A). In addition, the loss curves of the grain yield component showed higher slope (i.e., coefficient of yield loss) than the loss curves of the plant or the ear yield components at the two locations and for both the corn hybrids (Fig. 3A). Thus, the critical yield component in our study was grains regardless of year, corn hybrid (Cry1Ab or non-Bt) and location (with or without historical agricultural land use).

Within the critical yield component (i.e., grains), insects were responsible for the heaviest yield losses (Fig. 2B), and their loss curves showed the highest slope for both Cry1Ab and non-Bt corn at both the locations (Fig. 3B). More specifically, at location 1, Lepidoptera were the insects that best explained the grain losses as their attack produced loss curves with the highest slopes (Fig. 3C) and most contributed to the magnitude of the yield losses at this location (Fig. 2C). In contrast, Diptera best explained the variation in yield the losses at location 2 (Fig. 3C) although the magnitude of the yield losses due to their attack were relatively low, especially in the second year of the study (Fig. 2C). Hence, for both Cry1Ab and non-Bt corn hybrids, the key yield-loss factor at location 1 was the attack by Lepidoptera on corn grains, whereas at location 2, the key factor of grain yield loss was kernel destruction by Diptera. Not surprisingly, at location 2, the yield loss by dipteran larvae was not different between Bt and non-Bt corn hybrids ($P > 0.05$; mean \pm SE, 525 \pm 150 kg ha⁻¹). In contrast, at location 1, the Cry1Ab corn hybrid reduced the yield loss by the fall armyworm ($P < 0.05$; mean \pm SE, Cry1Ab: 87.6 \pm 35.5 kg ha⁻¹ vs. non-Bt: 184.0 \pm 52 kg ha⁻¹) but not by the corn earworm ($P > 0.05$, mean \pm SE, 1419 \pm 423 kg ha⁻¹) (see also Table 1).

Predaceous arthropods recorded during our study were the earwig *Doru luteipes* (Scudder) (overall mean \pm SE across year, location, and corn hybrid = 6.54 \pm 0.76 individual plot⁻¹), syrphid flies (1.03 \pm 0.20), ants (0.79 \pm 0.17), anthicoid bugs (0.52 \pm 0.13), spiders (0.26 \pm 0.03), anthicid beetles (0.19 \pm 0.15), carabid beetles (0.17 \pm 0.03), ladybeetles (0.17 \pm 0.03), rove beetles (0.07 \pm 0.02), big-eyed bugs (0.06 \pm 0.02), cantharid beetles (0.04 \pm 0.01), *Chrysoperla* lacewings (0.03 \pm 0.01), dolichopodid flies (0.02 \pm 0.01), soldier bugs (0.02 \pm 0.01), and braconid parasitoids (0.14 \pm 0.04). Importantly, the abundance of these arthropods were not significantly different between Cry1Ab and non-Bt corn hybrids ($F = 3.46$; $df = 1, 52$; $P = 0.068$), although they seem higher at location 2 than at location 1 in both years (Fig. 4).

Discussion

Although some researchers recognize the importance of understanding the components of yield losses for crops (Head et al. 2005, Bacci et al. 2006, Picanço et al. 2007, Hutchison et al. 2010, Pereira et al. 2017), as well as the impact of Bt corn on the infestation levels by target insects (Hutchison et al. 2010) and the abundance of natural

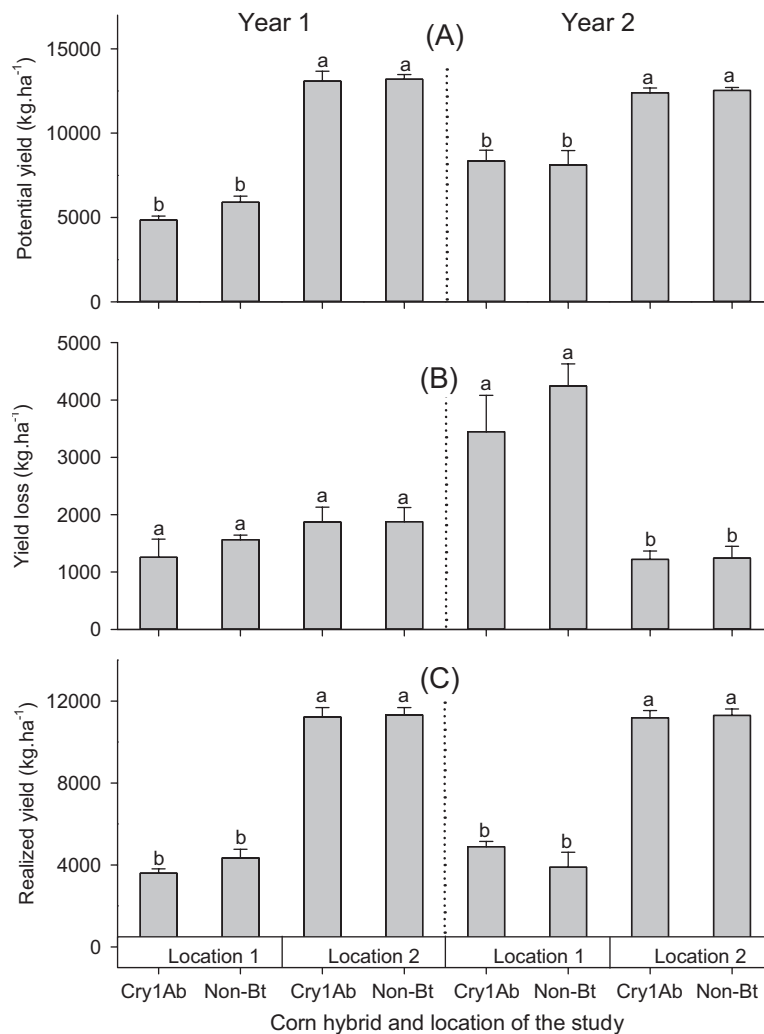


Fig. 1. Grain yield and loss for Bt Cry1Ab and non-Bt corn hybrids, recorded for 2 yr at two locations. (A) Potential yield at the beginning of the growing season, (B) yield loss occurred during the corn growth and development, and (C) realized yield at harvest. Within each year, means \pm standard errors followed by the same letter are not significantly different ($P > 0.05$) by analysis of variance (F test, $\alpha = 0.05$).

enemies (Fernandes et al. 2007), integrative studies considering all these aspects are scarce to date. Here we determined the critical components, key loss factors, and the potential role of natural biological control agents in fields of non-Bt and Bt Cry1Ab corn, which was the first transgenic Bt corn technology introduced in Brazil in 2007 aiming to provide some level of control against Lepidoptera (Mendes et al. 2011, Okumura et al. 2013).

Our study showed that regardless of Cry1Ab toxin in the corn hybrid, grain was the yield component regulating the intensity of the yield losses, and insects were the keys loss factors. These findings have implications for pest management suggesting that growers should use efficient control methods for kernel-attacking insect pests. Such measures may need to be applied to both Bt and non-Bt corn events, such as Cry1Ab corn, which here provided variable level of protection against kernel-attacking insects, similar to findings reported for fall armyworm in Brazil (Omoto et al. 2016, Sousa et al. 2016). We found that Lepidoptera and Diptera were the primary causes of losses in corn production (Figs. 2C and 3C). Although the Cry1Ab toxin (produced in MON 810 corn hybrids) is lepidopteran-specific, some caterpillar species (e.g., *H. zea* and *S. frugiperda*) were found attacking kernels in the field, a tissue that may not express Bt toxin concentration sufficient to control such noctuid pests. In

general, kernels and other ear tissues of Bt corn seem to produce low concentration of Cry toxin (i.e., 0.4–0.7 $\mu\text{g g}^{-1}$ dry matter) (Sears et al. 2001, Mendelsohn et al. 2003, Nguyen and Jehle 2007). In addition, ~25% of corn kernels show a low load of alleles encoding the Cry toxin due to the allelic segregation during gametogenesis (Horner et al. 2003). Thus, integrated pest management of both Bt and non-Bt corn, particularly during early reproductive stages of corn is important to minimize yield losses. Furthermore, that distinct insect groups were associated with variation in yield losses at each location in our study illustrates the importance of pest identification and monitoring for sound decisions as the pest complex can be different from one location to another.

Pest management for insects that attack corn kernels is challenging as control measures must be adopted before the insect gets into the ear and damage the kernels. In this scenario, Bt corn hybrids may be a useful tool to control *H. zea*, but our results indicate that the level of Cry1Ab toxin produced in MON 810 corn tissues where the larvae feed (i.e., style-stigmas and kernels) may be insufficient to do so depending on the pest pressure. A similar result (i.e., fair control by Cry1Ab corn) was obtained for *S. frugiperda* larvae, which feed mainly on whorl leaves (in the vegetative stage) and can migrate to the ears (in the reproductive stage). This finding is not unexpected

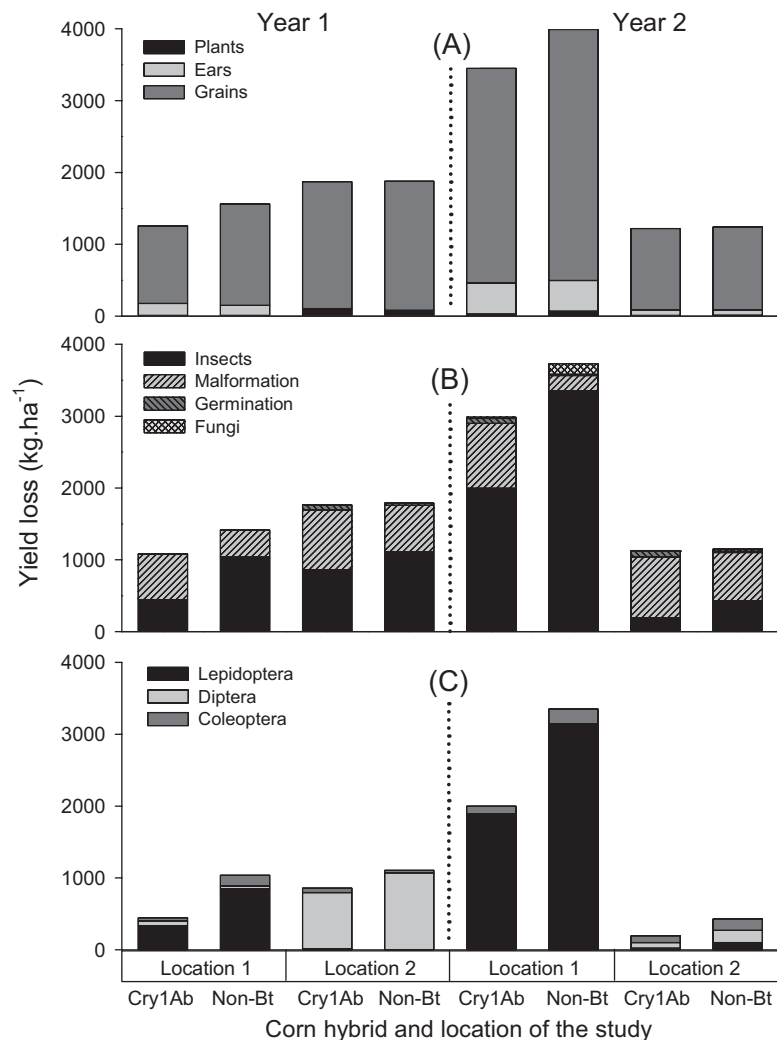


Fig. 2. Relative magnitude of the yield losses within each yield component and categories of loss factors recorded in fields of two corn hybrids, Bt Cry1Ab and non-Bt, in 2 yr at two locations. (A) Relative contribution of each yield component to the overall loss, (B) main categories of loss factors, and (C) insect groups accounting for the yield loss.

because Cry1Ab corn does not meet the high-dose condition for fall armyworm (i.e., >99.9% larval mortality) (Sousa et al. 2016), such that high proportion of the larvae are expected to survive on Cry1Ab corn (Sousa et al. 2016) and can attack the ears. Likewise, the infestation by *Euxesta* sp. in ears of Cry1Ab corn and non-Bt corn was expected due to the lepidopteran-specific toxicity of the Cry1Ab toxin, such that the silk fly larvae are not considered target of control by Cry1Ab corn. All these factors help explain why *H. zea*, *S. frugiperda*, and *Euxesta* sp. were key loss factors in our study. The implications are that other control measures such as biological and chemical control must be considered for proper management of these insect pests in Cry1Ab corn.

Most likely, the difference in corn yields between the two locations studied (Fig. 1A and C) was influenced by the local soil fertility, which was better and had lower Al³⁺ levels at location 2 (where annual crops were grown every year) than at location 1 (where there was no historical agricultural land use). High Al³⁺ levels in the soil are known to affect root growth and development, resulting in poor absorption of water and nutrients (Giannakoula et al. 2008). Interestingly, the grain yield of the Cry1Ab and non-Bt corn hybrids was similar at both locations (Fig. 1C), indicating that the physiological cost associated with transgene expression or acquisition of

novel traits (Baucom and Mauricio 2004, Robert et al. 2013) seems absent in Cry1Ab corn, or that, at least, it did not affect yield.

The same species of predaceous arthropods were observed in Cry1Ab and non-Bt corn hybrids, in which there was no detectable impact in their abundance, corroborating reports for other Bt corn hybrids (Fernandes et al. 2007; Meissle and Romeis 2009, 2012; Romeis et al. 2013). Importantly, the earwig *D. luteipes* was the most abundant predator (Fig. 4A), and its population density was significantly correlated ($P < 0.05$) with yield losses (i.e., infestation) by Lepidoptera. This finding is consistent with other reports, which recognize earwigs as the primary natural enemies of eggs and early instar larvae of both *S. frugiperda* and *H. zea* (Reis et al. 1988, Cruz et al. 1995, Cruz and Oliveira 1997). Other known natural enemies were also observed in our study (e.g., *Chrysoperla* sp., Carabidae, Staphilinidae, etc., Fig. 4A), consistent with the expectation of diverse entomofauna in tropical climates.

Regarding the main pests targeted by Bt corn (i.e., Lepidoptera), we found that the MON 810 corn did reduce the yield loss (i.e., damage) by *S. frugiperda* and *H. zea* in three of four field experiments, but the realized yield of grains at harvest were not different between the Bt and non-Bt corn hybrids. This is most likely because the infestation level and damage by lepidopterans was not severe enough and

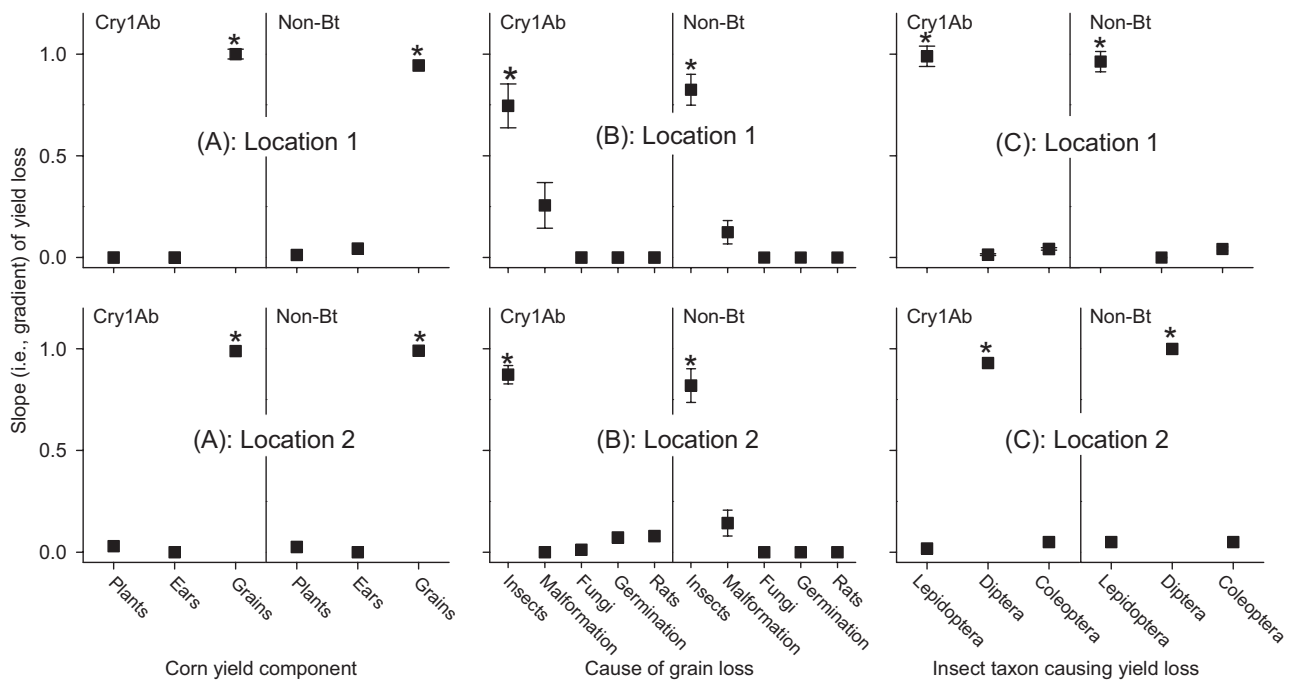


Fig. 3. Determining the critical yield component and key loss factors in two corn hybrids, Bt Cry1Ab and non-Bt. (A) Critical yield component, (B) key loss factors within the critical yield component, (C) insect taxon that were key factors in the yield losses at each location. Data are estimates of the slope \pm standard error of the line describing the relationship between the losses in a yield component or from loss factor and the total yield loss (in a logarithm scale). *The yield component or loss factor that showed the highest slope ($P < 0.05$, two-tailed t -test).

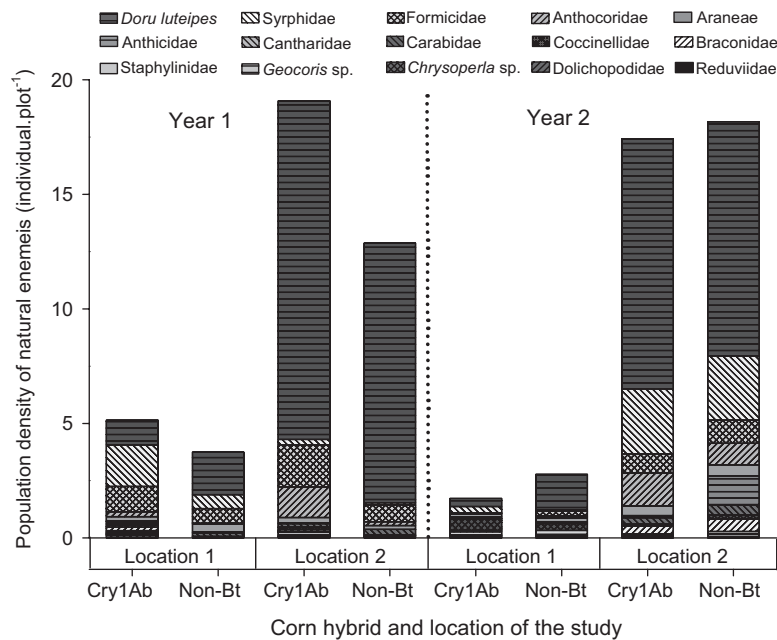


Fig. 4. Diversity and abundance of predaceous arthropods (i.e., natural enemies) during the reproductive stage of two corn hybrids, Bt Cry1Ab and non-Bt, recorded for 2 yr at two locations. Shown is mean population density of individuals of each species that was recorded in 10 plants per plot of 200 m² during 5 wk, starting in the silking stage.

the occurrence other yield-loss factors that are not affected by Bt corn (Table 1). The MON810 event was the first Bt corn event introduced in Brazil against *S. frugiperda* and *H. zea* (Mendes et al. 2011, CTNbio 2017). Some studies have shown that Cry1Ab corn reduced fall armyworm and corn earworm growth and survival (Williams et al. 1997, 1998; Bokonon-Ganta et al. 2003; Abel and Pollan 2004; Buntin 2008; Sousa et al. 2016), although field resistance evolution

in some *S. frugiperda* populations has been reported (Omoto et al. 2016). Our data suggest that when using Cry1Ab corn, other integrated pest management practices should be adopted to minimize yield losses by fall armyworm, corn earworm, and other insect pests in corn. These practices may include judicious interventions with chemical applications while trying to preserve the natural biological control (Campos et al. 2011), which in Neotropical corn fields

is provided by earwigs and other natural enemies (Cruz et al. 1995, Cruz and Oliveira 1997, Mendes, et al. 2012, Frizzas et al. 2014, Leite et al. 2014).

In summary, by using a modified life-table approach, our study showed that grain was the yield component most associated with the yield losses in both Cry1Ab and non-Bt corn hybrids and that insect herbivore attacks on the ears of *H. zea*, *S. frugiperda*, and *Euxesta* spp. were the key yield-loss factors. The Bt corn hybrid reduced losses from its target insects although their infestation level and damage may not have been severe enough to affect yield, resulting in similar overall corn yield in the Bt and non-Bt corn hybrids. There was no detectable impact of the Cry1Ab corn on the abundance and diversity of natural enemies during the growing season. These results indicate the need for integration of pest management practices to effectively minimize losses by kernel-destroying insects during corn reproductive stages when growing non-Bt or certain low-dose Bt corn cultivars for fall armyworm and corn earworm, such as those producing Cry1Ab or other Cry toxins.

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