

Biological Control and Crop Protection

Does *Bt* maize cultivation affect the non-target insect community in the agro ecosystem?


 Daniela Chaves Resende^a, Simone Martins Mendes^{a,*}, Rosangela C. Marucci^b,
 Alessandra de Carvalho Silva^c, Mônica Matoso Campanha^a, José Magid Waquil^d
^a *Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil*^b *Universidade Federal de Lavras, Lavras, MG, Brazil*^c *Embrapa Agrobiologia, Seropédica, RJ, Brazil*^d *Consulting and Training in Integrated Pest Management, Sete Lagoas, MG, Brazil*

ARTICLE INFO

Article history:

Received 16 December 2014

Accepted 20 November 2015

Available online 19 December 2015

Associate Editor: Mário A. Navarro Silva

Keywords:

Biosafety

GM maize

Tri-trophic interactions

Integrated pest management

ABSTRACT

The cultivation of genetically modified crops in Brazil has led to the need to assess the impacts of this technology on non-target species. Under field conditions, the potential effect on insect biodiversity was evaluated by comparing a homogeneous corn field with conventional and transgenic maize, expressing different *Bt* proteins in seven counties of Minas Gerais, Brazil. The richness pattern of non-target insect species, secondary pests and natural enemies were observed. The results do not support the hypothesis that *Bt* protein affects insect biodiversity. The richness and diversity data of insects studied were dependent on the location and other factors, such as the use of insecticides, which may be a major factor where they are used.

© 2015 Sociedade Brasileira de Entomologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Transgenic strategies for protecting crops against pests depend on the transfer and expression of defense genes to the crop species of interest. Among the most widely known and studied examples of induced resistance are those based on the use of the delta-endotoxin of the bacterium *Bacillus thuringiensis* Berliner, 1915, also known as *Bt* crops. This bacterium occurs naturally in soil and has the ability to form crystal proteins during the stationary and/or sporulation phase (Vasconcelos et al., 2011). After ingestion and solubilization of the crystals in the midgut of the insect, its degradation occurs from the action of proteases, releasing delta-endotoxins or Cry proteins, which adhere to specific receptors (Carneiro et al., 2009).

Bt toxins have high specificity, both for specific receptors in the gut and for the degradation of protein crystals by the alkaline pH in susceptible species. For decades, *Bt* bio-pesticides have been used for mosquito and insect pest control in agricultural and reforestation areas, and there have been no reports of adverse effects related to their use. However, there is at least one important difference between the *Bt* bio-insecticide and *Bt* transgenic

plants. The first case deals with a mixture of spores and crystals, sprayed on plants, and they must be activated in the gut of the insects, whereas in genetically modified (GM) plants, the protein is produced already activated in its toxic form. Thus, the question concerns those herbivorous insects that do not provide suitable conditions in their digestive tract to activate the proteins present in the bio-insecticides: may they still be affected by the toxin of the *Bt* transgenic plant, if they have specific receptors (Fontes et al., 2003)?

The commercial introduction of GM crops has led to the need to assess the possible impacts of this technology on the environment, and among the likely undesirable impacts are the effects on non-target organisms. Some studies have indicated possible toxic effects of *Bt* insecticidal proteins on non-target species, including other herbivores, scavengers, predators, parasitoids and soil fauna (Hilbeck et al., 1998; Losey et al., 1999; Schuler et al., 1999; O'Callaghan et al., 2005; Romeis et al., 2014). However, most of these studies tested the effect of these proteins on the species in unnatural conditions, not considering, for example, ecological interactions and the actual level of exposure of sensitive stages under natural conditions (Dale et al., 2002). More studies, considering multivariate systems and exposure conditions, similar to those present in the field, may provide more realistic information about the harmful effects of *Bt* crops on non-target species (for example, see Sears et al., 2001).

* Corresponding author.

E-mail: simone.mendes@embrapa.br (S.M. Mendes).

In addition, a number of studies have shown the impact in some specific cases. Hilbeck et al. (1998) reported that Cry 1 Ab-producing *Bt* maize and pure Cry 1Ab protein harmed larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae), but in this review, Romeis et al. (2014) show that there is sufficient information available today to conclude that *Bt* maize containing Cry 1Ab does not harm *C. carnea*. These authors discuss the necessity to develop conceptual field models, which should be based on properly designed studies that can be reproduced with a minimal probability of false positives or negatives. Thus, studies in the field should be focused.

With regard to natural enemies – key species within agro ecosystems that provide the biological pest control service – *Bt* plants could affect them directly, by the insect feeding on plant parts that express the protein (as in the case of predatory wasps and parasitoids that feed on pollen) or indirectly, by the use of prey that have fed on the transgenic plants (Pires et al., 2003; Frizzas and Oliveira, 2006). The search for prey in parasitoid species may occur associated with the perception of volatiles produced by plants as a function of herbivory, which also represents a source of impact, if GM plants have their attractiveness modified (Schuler et al., 1999). For any event, the actual reduction of the predated populations, due to the presence of the insecticidal protein in the GM plant, per se, may represent an impact on the population structure of the species of parasitoids and predators (White and Andow, 2005). From an environmental point of view, one possible advantage of the use of GM maize would be a reduction in insecticide applications – especially the broad spectrum active ingredient – since the effect of these can be more impactful on the persistence of the insect community (Dively, 2005; Naranjo, 2005).

Studies of *Bt*-transgenic crops have revealed that exposure to Cry proteins varies widely among different herbivore feeding guilds and species (Raybould et al., 2007; Romeis et al., 2009). Arthropods such as predators or parasitoids are mainly exposed to the plant-produced toxins when preying on or parasitizing herbivores that have fed on GM crops. There is evidence that the concentration of the arthropod-active compound is usually diluted as it moves up the food chain and does not accumulate (Romeis et al., 2009; Meissle and Romeis, 2009, 2012). Despite any possible advantage associated with the use of GMOs, the commercial release of these organisms is preceded by safety assessment studies carried out in each case. In Brazil, the National Biosafety Technical Commission (CTNBio) is in charge of the safety assessment of GMO crops. Regarding environmental risk assessment, few data under field conditions are available, so more research is needed to support effective models to anticipate potential changes in the agro ecosystem. Capalbo et al. (2009) emphasize that the Brazilian system does not require a specific evaluation process, which allows the use of any organism model, as long as the choice is described and justified. For scientific development, the continuous process of analysis and selection

highlights the need for ex post-release monitoring of *Bt* risk and impacts on the non-target community.

However, since GM crops represent a recent technological innovation and a novel evolutionary strategy, it is essential to maintain a process of continuous monitoring and evaluation of its efficacy and effects on the environment, especially independent posteriori risk assessments (Bauer-Panskus and Then, 2014). Thus, the aim of this study was to assess possible impacts of *Bt* maize on the insect biodiversity present in the agro ecosystem in different regions of Minas Gerais, comparing corn-fields growing conventional and transgenic maize, expressing different *Bt* proteins. The working hypothesis was that the presence of the *Bt* proteins does not affect the richness and diversity of insects present in crops.

Material and methods

Collection of biological material

This work involved monitoring the incidence of *S. frugiperda* – the primary target pest of maize – infesting the whorls and ears, and the insect community on conventional and *Bt* maize expressing different proteins, in seven different counties of Minas Gerais (Table 1).

In order to balance the technological level used in each corn field from different sampling areas, samples were collected from crop areas of more than 350 ha of maize with expected productivity around 200 bags/ha. To enable comparison of the insect community, collections from cornfields cultivated with conventional and *Bt* maize, expressing different proteins, were conducted. The collections were made in November and December of 2010. The crop field with conventional maize received three insecticide applications and the *Bt* maize received none.

The collection of biological material was performed in a systematic way in order to enable comparison of the richness and diversity observed on conventional and *Bt* maize. In each sample cornfield, three sampling points were selected and used as replication. At each sampling point, using the method proposed by Waquil (1997), whorls, ears and tassels of 10 randomly chosen plants were collected. The collected material was taken to the Embrapa Maize and Sorghum laboratory, in Sete Lagoas, MG. Insects found in the collected material were stored in 70% ethanol, separated and identified using bibliographic material available and with the assistance of specialists in different groups. The material was identified, when possible, at species level.

Statistical analyses

To evaluate the cultivation effect of *Bt* maize (different proteins) on the abundance of *S. frugiperda*, variance analyses were performed on two factors, considering the effect of treatment (maize

Table 1

Locations of conventional and *Bt* maize expressing proteins studied in different counties of the State of Minas Gerais.

County	State region	Coordinates	<i>Bt</i> protein
Três Corações	South/Southwestern Minas	21°41'41" S, 45°15'19"	Cry1F, Cry1A105 + Cry2Ab2, Cry1Ab
Nazareno	Campo das Vertentes	21°12'57" S, 44°36'39"	Cry1F, Cry1A105 + Cry2Ab2, Cry1Ab
Iguatama	Western Minas	20°10'26" S, 45°42'39"	Cry1Ab, Cry1A105 + Cry2Ab2, Cry1F
Inhaúma	Metropolitan region of Belo Horizonte	19°29'27" S, 44°23'24"	Cry1F, Cry1Ab
Matozinhos	Metropolitan region of Belo Horizonte	19°33'28" S, 44°4'51"	Cry1F, Cry1Ab
Varjão de Minas	Northwestern Minas	18°22'40" S, 46°1'55"	Cry1F, Cry1Ab
Iraí de Minas	Minas Triangle/Alto Paranaíba	18°59'2" S, 47°27'39"	Cry1F, Cry1Ab

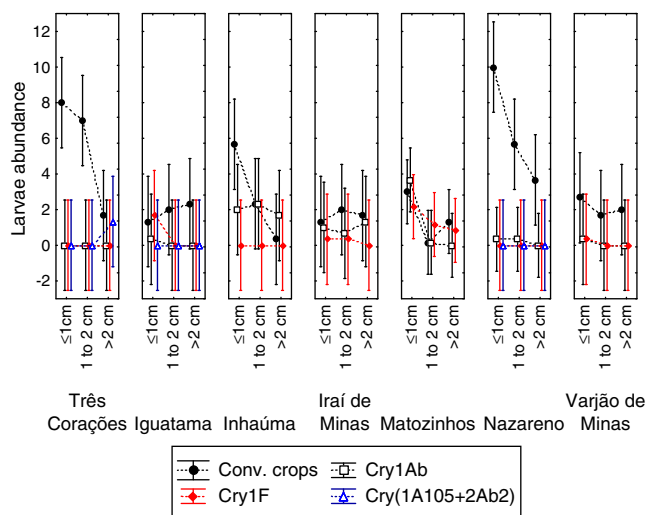


Fig. 1. Abundance of different sizes of larvae of *Spodoptera frugiperda* in whorls of conventional and transgenic maize (Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins) from cornfields of different counties in Minas Gerais. Bars represent a 95% confidence interval.

hybrid) and the size of the larvae, according to [Fernandes et al. \(2003\)](#). These analyses were performed separately for data collected in the whorl and ear and grouped at the location (county), since the experimental design was incomplete, because not all crop fields used the same hybrid seeds.

Species richness was estimated using the Jackknife procedure, since this method addresses the intrinsic defect of underestimation that occurs in this type of data ([Heltshe and Forrester, 1983](#)). The Jackknife procedure, being a re-sampling technique, provides in addition to species richness estimation, an estimate of the confidence interval, allowing a statistical comparison between different locations. To estimate the diversity, the Shannon–Wiener index ([Krebs, 1999](#)) was used. The EstimateS 8.0 software ([Colwell, 2000](#)) was used for the analyses.

To evaluate the effect of *Bt* maize cultivation on the estimated richness and diversity of secondary pests and natural enemies, variance analyses were performed on two factors, considering the effect of treatment (type of protein) and the type of organism. These analyses were performed separately for data collected in the whorl, ear and tassel and grouped at the location (county), since the experimental design was incomplete, because not all the crops used the same seed type. To facilitate interpretation of the results, data from all cornfields with different *Bt* maize crops are presented together in charts, thus considering the conventional and *Bt* treatments.

Regression analyses were also performed to evaluate if the richness of natural enemies present in the crop fields was related to the richness of secondary pests and if the total area sown in each cornfield affected the insect richness by location. To evaluate the insecticide spraying affected on the estimated insect richness, Paired *t*-test analyses were performed.

Results

Abundance of *S. frugiperda*

The abundance of *S. frugiperda* larvae of different sizes collected in whorl samples was dependent on the location ($N = 252$, $F = 1.56$, $p < 0.05$, [Fig. 1](#)). The abundance of *S. frugiperda* in the cornfield with *Bt* maize was lower in the three studied counties – Inhaúma, Três Corações and Nazareno. In Inhaúma, the difference occurred only for larvae with a body size smaller than 1 cm, in Três Corações,

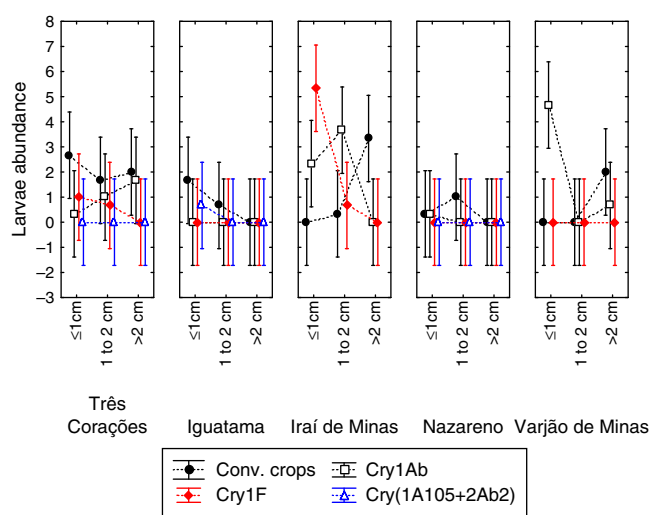


Fig. 2. Abundance of different size larvae of *Spodoptera frugiperda* in ears of conventional and transgenic maize (Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins) from cornfields of different counties in Minas Gerais. Bars represent a 95% confidence interval.

where the difference was for larvae smaller than 2 cm, and finally in Nazareno, where the conventional treatment showed higher abundance of *S. frugiperda* independent of larval size.

The treatment effects on frequency of the different larval sizes of *S. frugiperda* in the ears were significantly different among the sampling sites ($N = 162$, $F = 1.96$, $p < 0.01$, [Fig. 2](#)). In Três Corações, for larvae smaller than 1 cm, the abundance of *S. frugiperda* was higher in the cornfield with conventional maize. For larvae larger than 2 cm, the abundance of *S. frugiperda* was higher in the conventional cornfield than in the field with *Bt* maize, except in the *Bt* cornfield with Cry1Ab protein. In Iraí de Minas, for larval body size smaller than 1 cm in the cornfield with conventional maize, the abundance of *S. frugiperda* was lower than the in cornfield with transgenic maize. Moreover, in the cornfield with Cry1F protein, the abundance of *S. frugiperda* was higher than in the cornfield with Cry1Ab protein. For larval body size between 1 and 2 cm, the abundance of *S. frugiperda* was higher in the cornfield with Cry1Ab protein, whereas for larval body size greater than 2 cm, the conventional cornfield had quite a high abundance of *S. frugiperda*. And finally, in Varjão de Minas for larvae smaller than 1 cm, the cornfield cultivated with the *Bt* hybrid expressing Cry1Ab protein showed higher abundance of *S. frugiperda* than the other treatments, whereas for larvae larger than 2 cm, the conventional cornfield had a higher abundance of *S. frugiperda* than the cornfield with Cry1F transgenic maize.

Overall insect richness

The overall pattern of estimated richness of insects in the different plant parts of maize was distinct among the seven crop fields studied, and the results did not support the hypothesis of *Bt* proteins having a negative effect on insect richness.

Considering the insect community in the whorl, a reduction in richness was observed in the *Bt* cornfields, with hybrids expressing Cry1Ab protein from the counties of Nazareno ([Fig. 3](#)), Varjão de Minas ([Fig. 4](#)) and Iraí de Minas ([Fig. 5](#)). In Três Corações ([Fig. 6](#)) and Inhaúma ([Fig. 7](#)), the estimated insect richness in the cornfield with *Bt* maize was higher than in conventional maize. Comparing only the *Bt* cornfields in Três Corações and Iguatama, the transgenic maize with Cry2Ab2 and Cry1A105 proteins, the observed richness was lower in the first crop, only when compared with a cornfield expressing Cry1F protein. In Inhaúma and Nazareno,

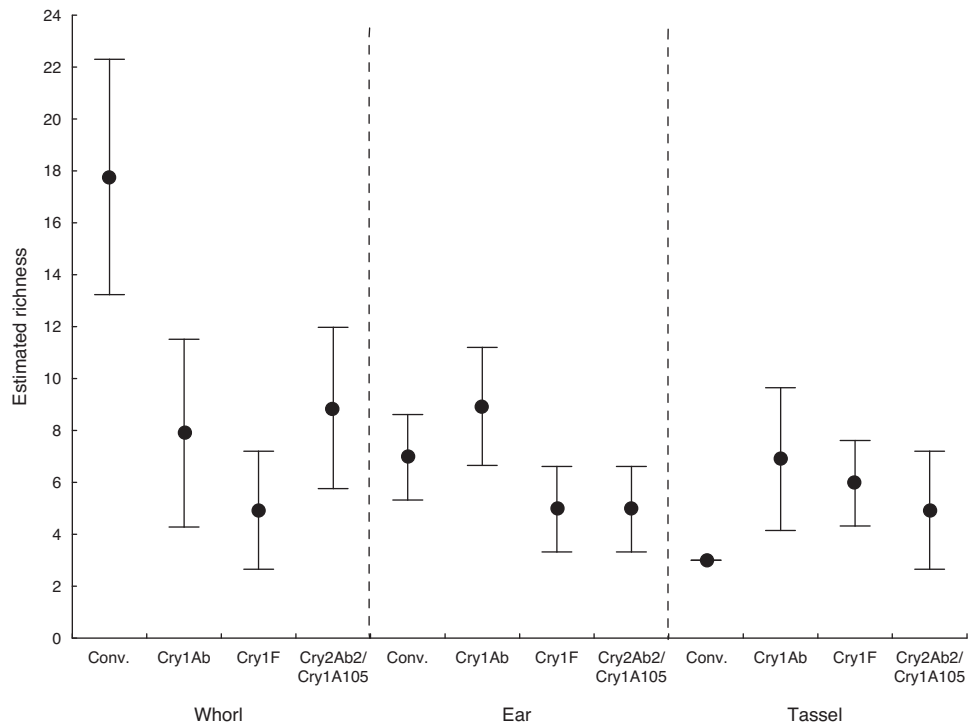


Fig. 3. Estimated richness of insects in the whorl, ear and tassel of conventional (Conv.) and transgenic maize (Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins) from Nazareno county, MG. Bars represent 95% confidence interval.

the estimated insect richness in a cornfield with Cry1F transgenic maize was lower than in the other locations with transgenic maize expressing the combined proteins Cry2Ab2 and Cry1A105.

The data from the ears showed a reduction in estimated richness of insects in the *Bt* corn field in Nazareno, except for the *Bt* (Cry1Ab) corn field (Fig. 3) and in Varjão de Minas, only in the cornfield with Cry1Ab protein (Fig. 4). In Três Corações (Fig. 6), the cornfield with conventional maize had a lower estimated richness compared to transgenic maize with Cry2Ab2 and Cry1A105 proteins. In

Iguatama (Fig. 8), only in the *Bt* (Cry1F) cornfield was the insect richness lower than in other treatments, whereas in Nazareno (Fig. 3), the *Bt* (Cry1Ab) cornfield presented higher richness than in other transgenic treatments.

Finally, considering the data from tassels, the estimated insect richness was lower in the conventional cornfield in Três Corações (Fig. 6), Iraí de Minas (Fig. 5), Nazareno (Fig. 3) and Varjão de Minas (Fig. 4) when compared to the richness observed in all *Bt* corn fields. In other crop fields, there was no difference in estimated

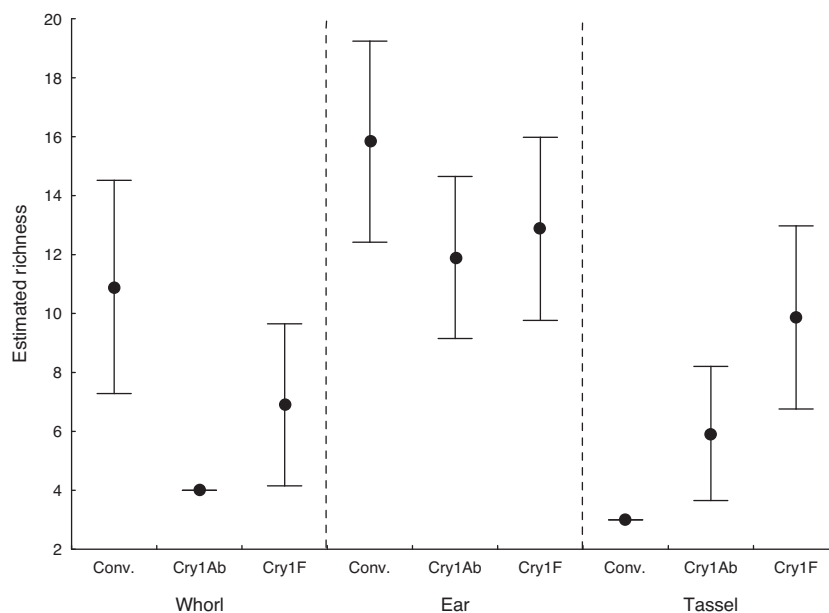


Fig. 4. Estimated richness of insects in the whorl, ear and tassel of conventional (Conv.) and transgenic maize (Cry1Ab and Cry1F proteins) from Varjão de Minas county. Bars represent 95% confidence interval.

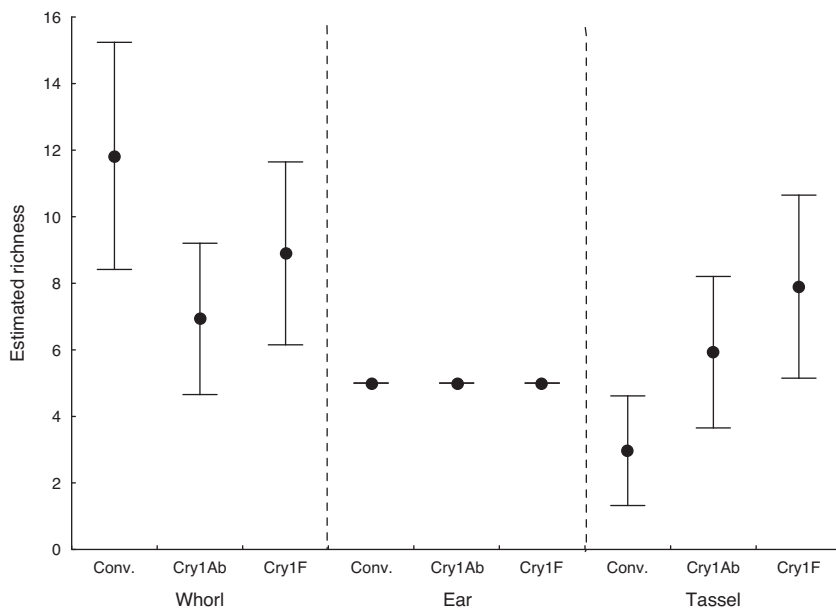


Fig. 5. Estimated richness of insects in the whorl, ear and tassel of conventional (Conv.) and transgenic maize (Cry1Ab and Cry1F proteins) from Iraí de Minas Gerais county. Bars represent 95% confidence interval.

insect richness between conventional and *Bt* cornfields. Comparing only the treatments with *Bt* (Cry1Ab) maize in Três Corações and Iguatama, the richness was higher than in the other treatments, whereas in Varjão de Minas the richness was higher than the other treatments in a *Bt* cornfield with Cry1F protein (see Fig. 9).

Richness of non-target insects: secondary pests and natural enemies

Considering the data from maize whorls, there was no interaction between the treatments' effect on the estimated richness

of secondary pests and natural enemies ($N = 155$, $F = 1.63$, $p < 0.05$, Fig. 10). It could be observed that in the cornfield from the three counties (Três Corações, Inhaúma and Matozinhos), the richness of secondary pests was higher on *Bt* than on conventional maize. In Iguatama, there was a reduction in the secondary pests' richness by growing *Bt* maize, while in Nazareno, both the secondary pests' and natural enemies' richness were higher in the conventional corn field (Fig. 10B).

Similarly, there was an interaction between treatment effects on diversity – estimated by the Shannon index – of secondary pests and natural enemies ($N = 155$, $F = 1.85$, $p < 0.01$, Fig. 11). It was possible

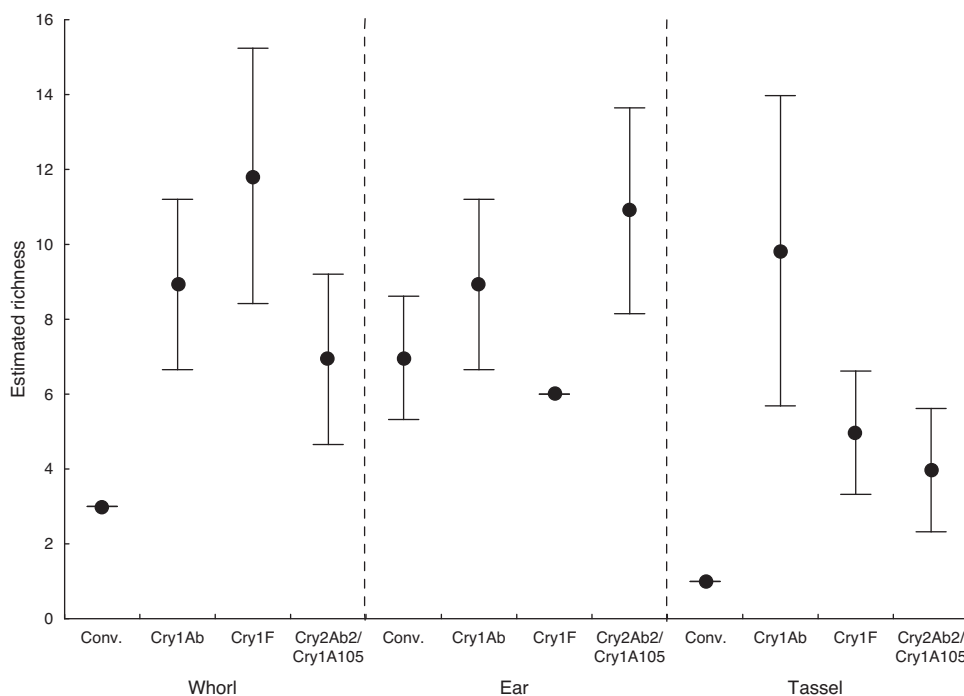


Fig. 6. Estimated richness of insects in the whorl, ear and tassel of conventional (Conv.) and transgenic maize (Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins) from Três Corações county, MG. Bars represent 95% confidence interval.

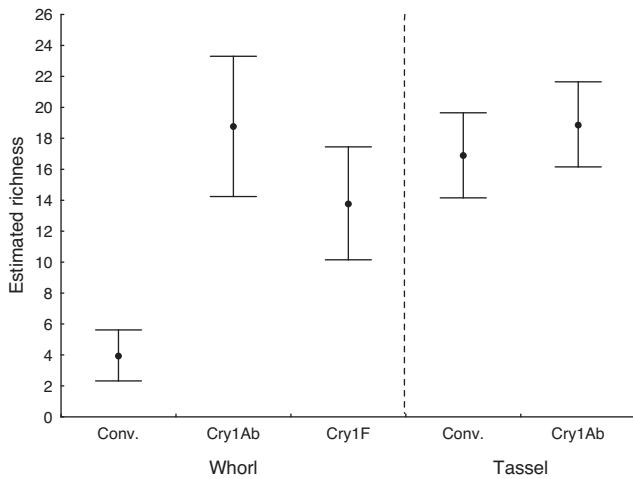


Fig. 7. Estimated richness of insects in the whorl and tassel of conventional (Conv.) and transgenic maize (Cry1Ab and Cry1F proteins) from Inhaúma county, MG. Bars represent 95% confidence interval.

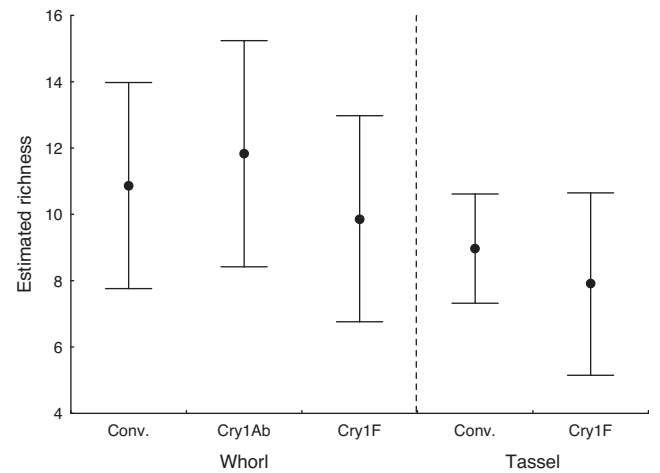


Fig. 9. Estimated richness of insects in the whorl and tassel of conventional (Conv.) and transgenic maize (Cry1Ab and Cry1F proteins) from Matozinhos county, MG. Bars represent 95% confidence interval.

to observe a reduction in the secondary pests' and natural enemies' diversity in crop fields with *Bt* maize, in Iguatama and Nazareno counties. In Varjão de Minas, there was a reduction only in secondary pests' diversity. In Três Corações and Matozinhos, in turn, we found an increase in the diversity of secondary pests in the *Bt* cornfield.

Regarding ears, there was also no interaction between the treatment effect on the estimated richness of different types of organisms and location ($N = 108$, $F = 2.35$, $p < 0.01$, Fig. 12). In four of the five crop fields studied, the pattern observed was similar, with no difference between the richness estimated in conventional and *Bt* cornfields, despite higher estimated richness of secondary pest species than the natural enemy. However, in Varjão de Minas, the estimated secondary pest richness was higher in the conventional

cornfield than in the *Bt* cornfield, while the natural enemies' estimated richness was higher in the *Bt* cornfield.

The treatments' effects on insect diversity – estimated by the Shannon index – was different between studied sites ($N = 108$, $F = 3.75$, $p < 0.001$, Fig. 13). In the cornfields in Três Corações, Iguatama and Iraí de Minas there were no differences in estimated diversity between the conventional and *Bt* cornfields. In the crop fields studied in Nazareno and Varjão de Minas, the estimated secondary pests' diversity was higher in the conventional cornfield and, moreover, in Varjão de Minas, the natural enemy diversity was higher in the *Bt* cornfield.

Finally, considering the richness on plant tassels, once again the treatment effects on the type of organisms was significantly dependent on the studied sites ($N = 132$, $F = 5.22$, $p < 0.001$, Fig. 14).

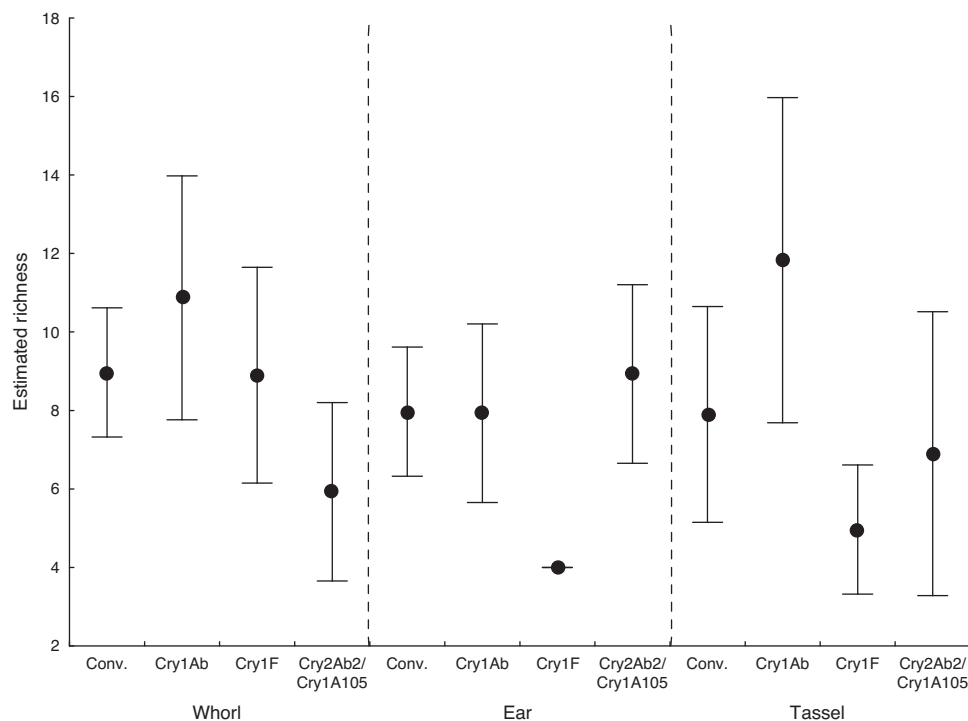


Fig. 8. Estimated richness of insects in the whorl, ear and tassel of conventional (Conv.) and transgenic maize (Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins) from Iguatama county, MG. Bars represent 95% confidence interval.

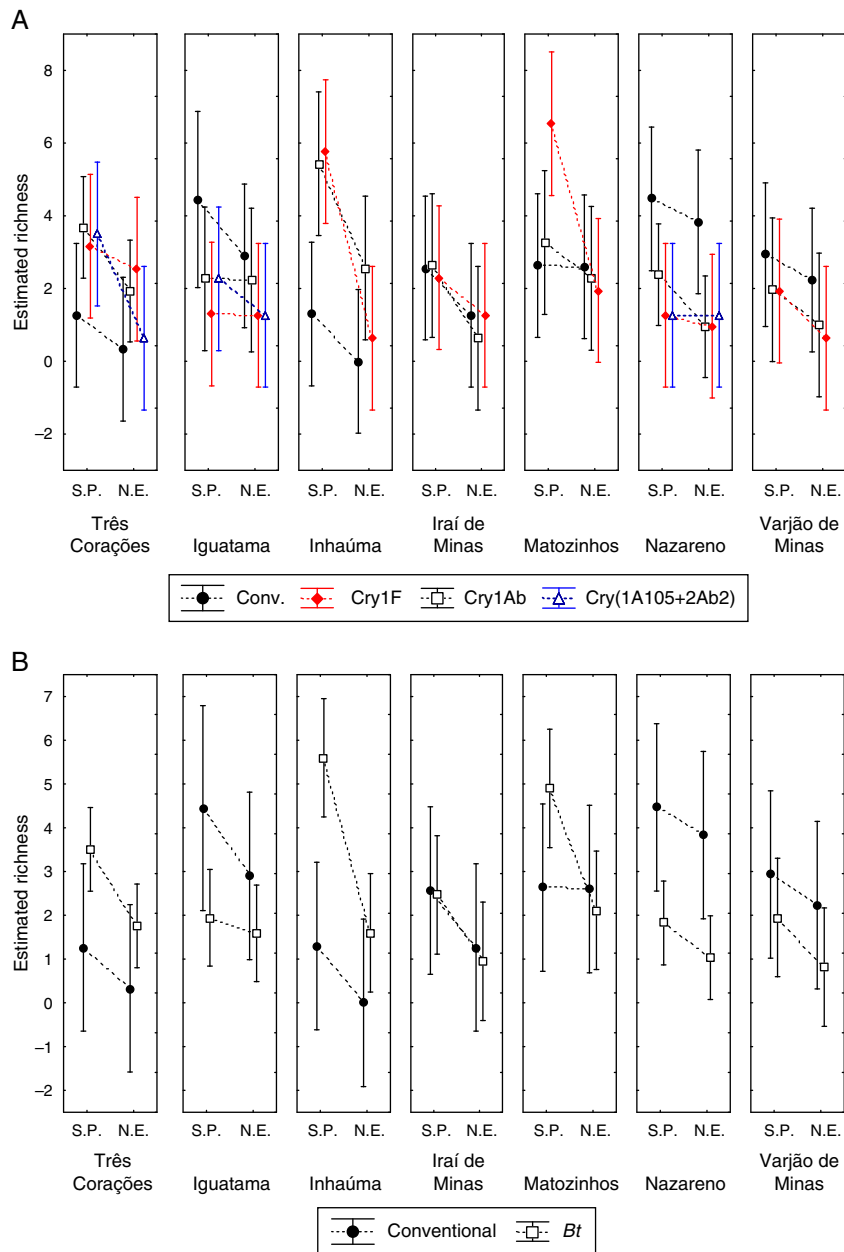


Fig. 10. Estimated richness of secondary pests (S.P.) and natural enemies (N.E.) in conventional and transgenic maize whorls for Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins (A) and estimated richness in conventional maize and *Bt* maize (B) in different counties in Minas Gerais. Bars represent 95% confidence interval.

In the cornfields from Três Corações, Iguatama, Iraí de Minas and Varjão de Minas there were no significant differences between conventional and *Bt* cornfields either for secondary pests' or for natural enemies' estimated richness. In Inhaúma and Nazareno, the estimated richness of secondary pests was higher in the *Bt* than in the conventional cornfield, while in Matozinhos both the richness of secondary pests as well as natural enemies was higher in the conventional cornfield.

Again, there was an interaction between treatment effects on the estimated diversity of organisms on plant tassels and study location ($N = 132$, $F = 5.44$, $p < 0.001$, Fig. 15). In six crop fields, there was no difference in estimated diversity, either for secondary pests or for natural enemies, between conventional and *Bt* corn fields. However, it is worth noting that in Inhaúma, there was a considerable increase in the diversity of natural enemies. In Matozinhos, in turn, the same pattern was not observed since the diversity, both

of secondary pests as well as natural enemies, was higher in the conventional than in the *Bt* cornfield.

Among the insect species observed in different studied parts of maize plants, *Carpophilus* sp. (Coleoptera: Nitidulidae) and *Euxesta* sp. (Diptera: Ulidiidae) were the species that showed an apparent reduction in abundance in the *Bt* cornfield. Among the predators, the earwig *Doru luteipes* (Scudder, 1876) (Dermaptera: Forficulidae) and minute pirate bug *Orius insidiosus* (Say, 1832) (Hemiptera: Anthocoridae) showed considerable fluctuation in abundance among the different treatments evaluated (Table 2).

Local characteristics

The effects of *Bt* maize on the overall richness and diversity of insects appear to be dependent on the geographical location and

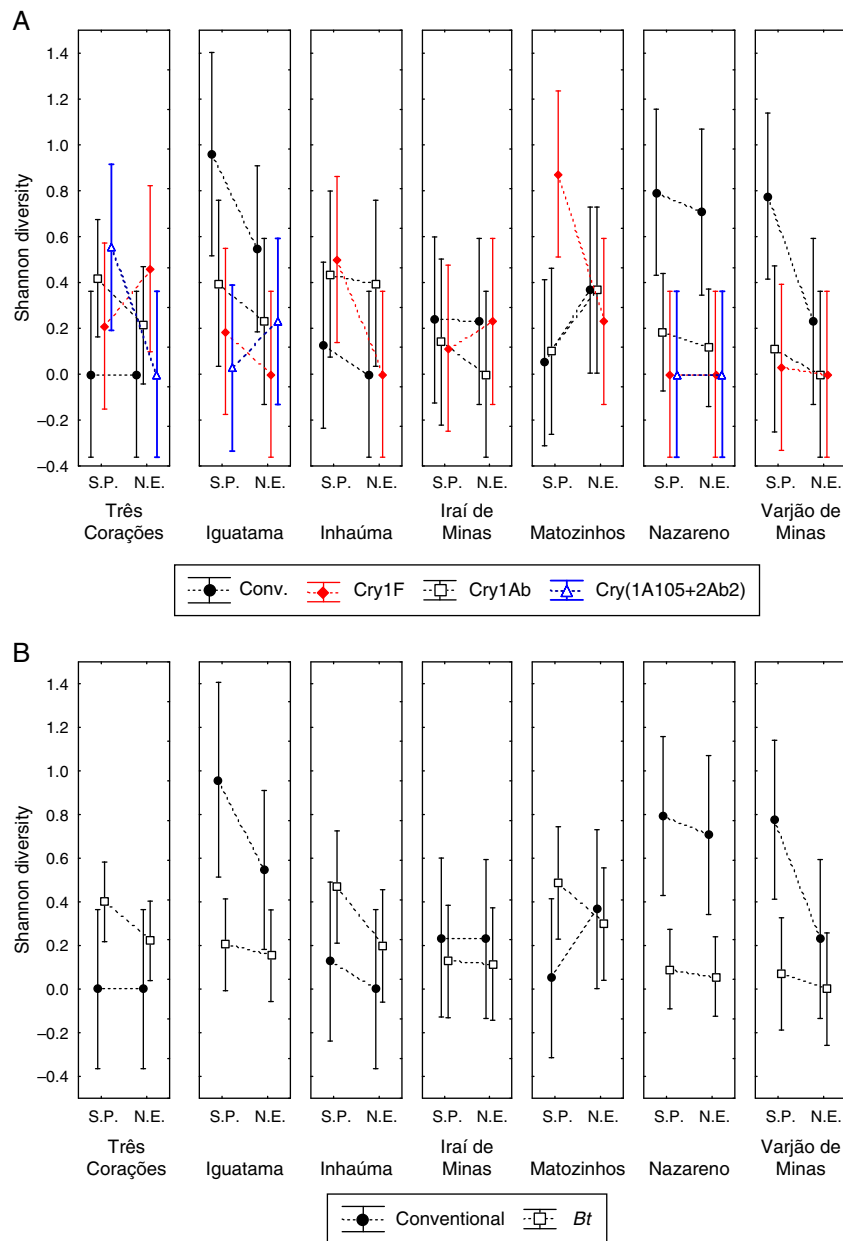


Fig. 11. Estimated diversity secondary pests (S.P.) and natural enemies (N.E.) in conventional and transgenic maize whorls for Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins (A) and estimated diversity in conventional maize and *Bt* maize (B) in different counties in Minas Gerais. Bars represent 95% confidence interval.

crop management in the agro ecosystem. So there is an indication that other factors, such as spraying insecticides on the cornfield or not, may exert a stronger influence on this process.

The estimated richness of natural enemies, however, appears to be influenced by the richness of secondary pests, in spite of the possibility of a limited number of samplings to show this relationship ($R^2=0.51$, $b=0.71$, $t=2.28$, $p=0.07$, Fig. 16). Among all the sites studied, only Inhaúma is further removed from the proposed model, presenting a higher richness of natural enemies than would be expected based on the number of secondary pests, so that, excluding this analysis location, this relationship was confirmed ($R^2=0.75$, $b=0.86$, $t=3.46$, $p<0.05$).

The total area sown in each cornfield did not influence the estimated richness of insects ($R^2=0.00$; $b=0.00$; $t=0.009$, $p=0.99$). Similarly, there was no effect of the use of insecticides on the estimated insect richness ($N=7$, $t=0.48$, $p=0.64$), for secondary

pests ($N=7$, $t=0.31$, $p=0.76$) or natural enemies ($N=7$, $t=1.63$, $p=0.15$).

Discussion

The effect of the use of *Bt* maize controlling *S. frugiperda* was dependent on the *Bt* event considered and the location studied during all sampling times. These data suggest that the species community may respond to the presence of *Bt* maize linked to interactions among different factors like local adaptations that generate the combinations of different populations (Busato et al., 2004, 2005). For samples from the whorl, only three of the seven cornfields studied showed enough infestation to discriminate the treatments that reduced the abundance of larvae on *Bt* maize. Furthermore, in these cornfields the abundance of larvae of small size (up to 2 cm) in conventional maize was significantly greater

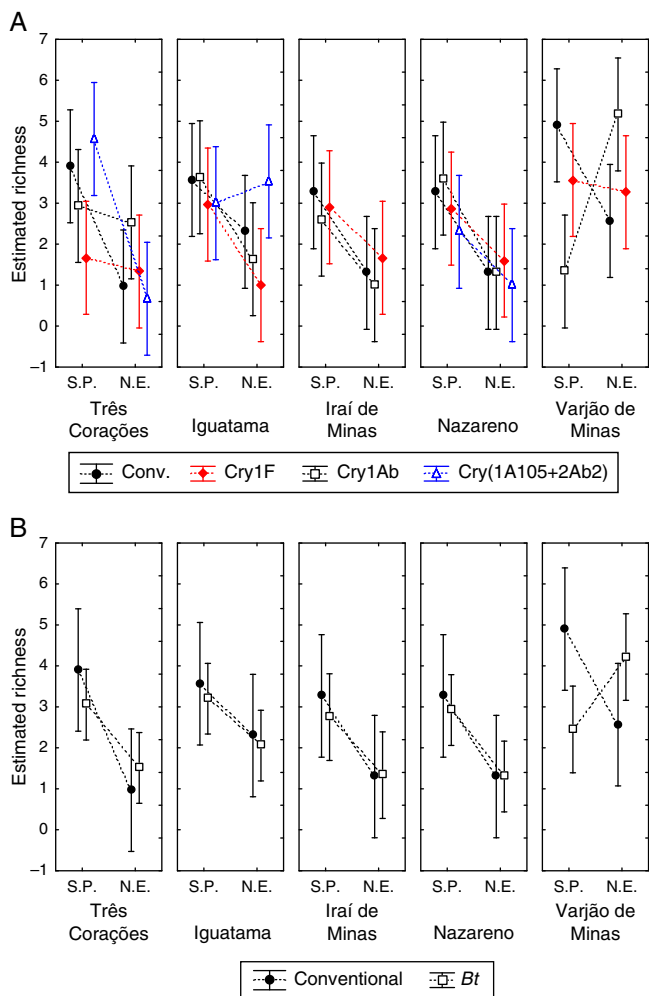


Fig. 12. Estimated richness of secondary pests (S.P.) and natural enemies (N.E.) in ears of conventional and transgenic maize for Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins (A) and estimated richness in conventional maize and Bt maize (B) in different counties in Minas Gerais. Bars represent 95% confidence interval.

than the abundance of larger larvae. According to [Waquil et al. \(2004\)](#) and [Mendes et al. \(2011\)](#), the use of Bt maize often does not lead to the immediate death of caterpillars, but reduces their development, making the insect more vulnerable to biotic and abiotic mortality factors. Another hypothesis for this situation may be associated with the results encountered by [Paula et al. \(2014\)](#), indicating that the lepidopteran *Chlosyne lacinia*, in experimental conditions, transferred to descendants the Bt insecticidal protein, increasing the mortality and developmental time. It is noteworthy that all conventional cornfields were sprayed with insecticides, which shows once again that different locations may present different difficulty levels regarding the control of this pest.

The overall pattern of estimated richness of secondary pests and natural enemies was very different among the seven cornfields studied, and the variation observed was not consistent with the hypothesis of the Bt proteins' effect on the structure of the insect community. In many cases, the insect richness estimated for conventional and Bt cornfields was not significantly different or was lower than on Bt maize. Again, considering that conventional cornfields underwent insecticide spraying, it is likely that the low insect richness in these fields is the result of this impact. However, although other studies have already shown that the impact of

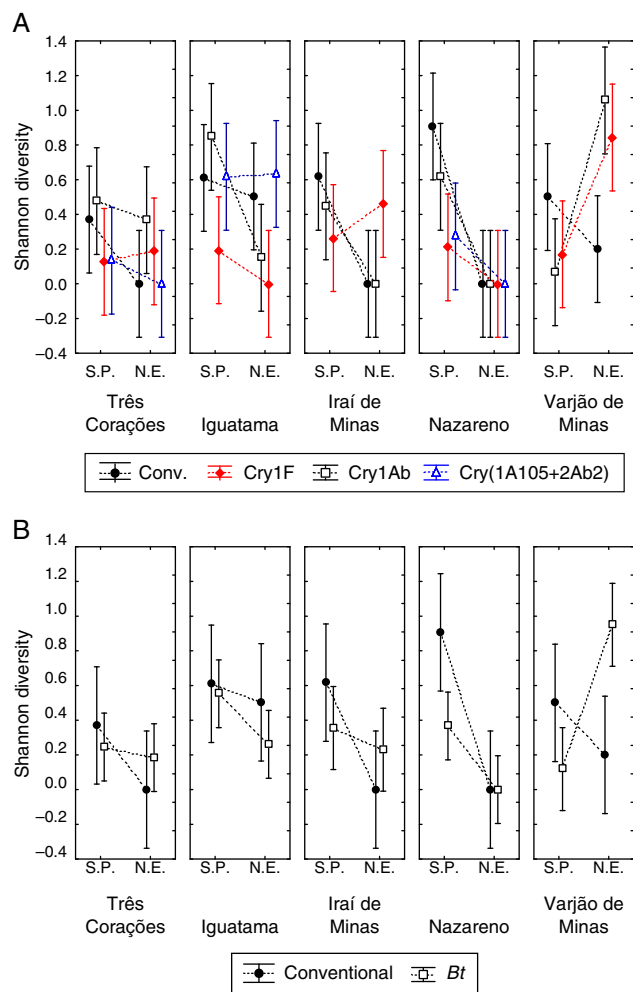


Fig. 13. Estimated diversity of secondary pests (S.P.) and natural enemies (N.E.) in ears of conventional and transgenic maize for Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins (A) and estimated diversity in conventional maize and Bt maize (B), in different counties in Minas Gerais. Bars represent 95% confidence interval.

insecticide use may be stronger on the structure of insect communities than the impacts of transgenic Bt crops, in the present study, the estimated insect richness was not significantly affected by insecticide use on the studied cornfields ([Dively, 2005](#)). Since all these croplands are already occupied by Bt maize for much of their commercial production, it can be seen that there has been a reduction in the use of these pesticides. In only three of the seven crop fields studied have broad-spectrum insecticides been used and, in such cases, only on conventional maize cultivars. Moreover, in only four of these cornfields was insecticide sprayed more than once, with a maximum of three applications (in Inhaúma and Três Corações). This represents a low use of insecticide if compared with other regions, where it is common to have up to eight sprayings during a crop season.

The abundance of *Carpophilus* sp. was reduced in Bt crops, regardless of the plant part. This result is consistent with other studies showing that damage reduction in Bt cultivation – from the target pest – results in a lower colonization by saprophagous species ([Dively, 2005](#)). Species of Diptera, genus *Euxesta*, may also undergo this effect, because oviposition of this species appears to be stimulated by the previous plant damage occurrence, which happens less in Bt maize fields ([Cruz et al., 2011](#)). The apparent fluctuations in the abundance of predators among the different treatments studied

Table 2

Total abundance of insect by species (herbivores and natural enemies) sampled in plants (whorl, ear and tassel) of conventional and transgenic maize expressing Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins from different Minas Gerais counties.

Taxon	Conventional	Cry1Ab	Cry1F	Cry(1A105 + 2Ab2)
Whorl				
<i>Herbivores</i>				
<i>Dalbulus maidis</i> DeLong, 1923	4	3	2	1
<i>Peregrinus maidis</i> (Ashmead, 1890)	0	1	0	0
<i>Deois flavopicta</i> (Stal, 1854) and <i>Mahanarva</i> sp.	8	11	1	2
<i>Diabrotica speciosa</i> Germar, 1824	0	5	6	1
<i>Diabrotica viridula</i> Fabricius, 1801	1	0	1	0
Thrips (Thripidae)	1	1	0	0
<i>Lagria villosa</i> (Fabricius, 1781)	0	11	3	0
<i>Rhopalosiphum maidis</i> (Fitch, 1856)	688	1101	329	109
<i>Colaspis</i> sp.	20	33	9	0
Elateridae	2	0	0	0
<i>Euxesta</i> sp.	8	4	6	0
<i>Carpophilus</i> sp.	25	13	3	5
<i>Natural enemies</i>				
Reduviidae 1	1	1	2	1
Reduviidae 2	0	1	0	1
<i>Orius insidiosus</i> (Say, 1832)	2	0	1	0
Miridae	0	2	0	0
<i>Lebia</i> sp.	1	1	2	0
Anthicidae	1	0	0	0
<i>Callida scutellaris</i>	1	1	0	0
<i>Doru luteipes</i> Scudder, 1876	34	30	4	2
Chrysopidae	0	16	1	0
<i>Geocoris punctipes</i> (Say, 1832)	10	4	1	2
Ear				
<i>Herbivores</i>				
<i>Dalbulus maidis</i> DeLong, 1923	0	0	2	0
<i>Diabrotica speciosa</i> Germar, 1824	1	0	0	1
Thrips (Thripidae)	0	1	0	2
<i>Lagria villosa</i> (Fabricius, 1781)	0	1	0	1
<i>Rhopalosiphum maidis</i> (Fitch, 1856)	1574	593	2613	1382
<i>Colaspis</i> sp.	1	0	9	7
<i>Euxesta</i> sp.	105	49	54	73
<i>Carpophilus</i> sp.	229	183	59	49
<i>Natural enemies</i>				
Reduviidae 1	17	8	3	0
Reduviidae 2	0	1	1	0
<i>Nabis</i> sp.	1	1	0	0
<i>Orius insidiosus</i> (Say, 1832)	0	8	31	0
<i>Lebia</i> sp.	0	0	0	1
<i>Doru luteipes</i> Scudder, 1876	72	65	127	58
<i>Cycloneda sanguinea</i> (Linnaeus, 1763)	1	2	1	0
Chrysopidae	1	0	0	1
<i>Euborelia annulipes</i> (Lucas, 1847)	0	1	0	0
Tassel				
<i>Herbivores</i>				
<i>Dalbulus maidis</i> DeLong 1923	0	2	3	1
<i>Peregrinus maidis</i> (Ashmead, 1890)	0	4	0	0
<i>Diabrotica speciosa</i> Germar, 1824	45	136	1	1
<i>Diabrotica viridula</i> Fabricius, 1801	7	11	0	0
Thrips (Thripidae) sp1	0	0	1	0
Thrips (Thripidae) sp2	0	1	0	0
<i>Rhopalosiphum maidis</i> (Fitch, 1856)	2666	9166	342	600
<i>Colaspis</i> sp.	2	4	1	0
<i>Euxesta</i> sp.	1	0	0	0
<i>Carpophilus</i> sp.	77	53	22	0
<i>Natural enemies</i>				
Reduviidae sp. 1	7	7	0	0
Reduviidae sp. 2	2	0	0	0
<i>Nabis</i> sp.	1	0	0	0
<i>Orius insidiosus</i> (Say, 1832)	86	13	14	0
<i>Lebia</i> sp.	0	1	0	0
Anthicidae	0	1	0	0
<i>Callida scutellaris</i>	0	4	0	0
<i>Doru luteipes</i> Scudder, 1876	38	42	11	2
<i>Cycloneda sanguinea</i> (Linnaeus, 1763)	7	1	1	0
Chrysopidae	1	0	0	0
Cantharidae	2	0	0	0
<i>Euborelia annulipes</i> (Lucas, 1847)	1	4	0	0
Syrphidae	2	0	0	0
Lampyridae	1	0	0	0

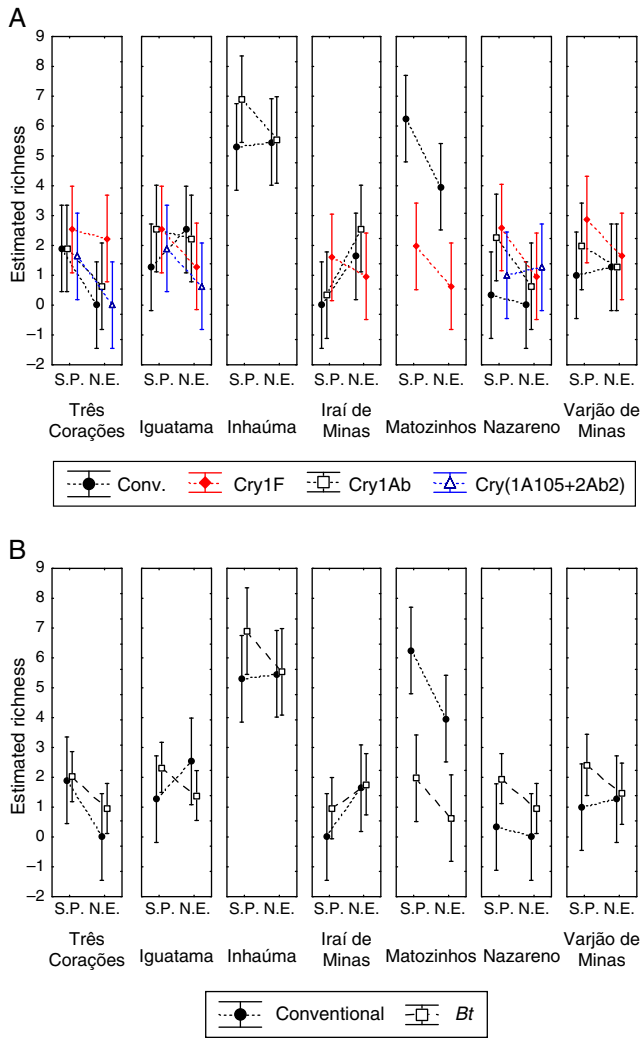


Fig. 14. Estimated richness of secondary pests (S.P.) and natural enemies (N.E.) in tassels of conventional and transgenic maize for Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins (A) and estimated richness in conventional maize and *Bt* maize (B), in different counties in Minas Gerais. Bars represent 95% confidence interval.

appears consistent with the variation observed in the abundance of other non-target herbivores, which is also consistent with results already reported in the literature, since predators – generalist natural enemies – seem to be less affected by the presence of *Bt* maize than parasitoids (Pilcher et al., 2005; Romeis et al., 2014).

The discussion on the impact or environmental risk associated with the use of genetically modified* crops in agriculture must start with the definition of the reference starting point (Frizzas and Oliveira, 2006) and be followed by monitoring as stated by CTNBio. This is because the use of transgenic plants is just one more method among various Integrated Pest Management (IPM) strategies available. Regarding community diversity, the richness of secondary pests was directly related with the richness of natural enemies. Also, the data and recent literature indicate no significant effect of *Bt* proteins on natural enemies. Various practices used in modern agriculture are associated with the decline of biodiversity in agro ecosystems, especially those related to intensive agriculture, such as monoculture, use of fertilizers and pesticides. The simplification of the landscape related to agriculture reduces the diversity of fauna and the structure of communities associated with agro ecosystems and the findings of this study reinforce this idea.

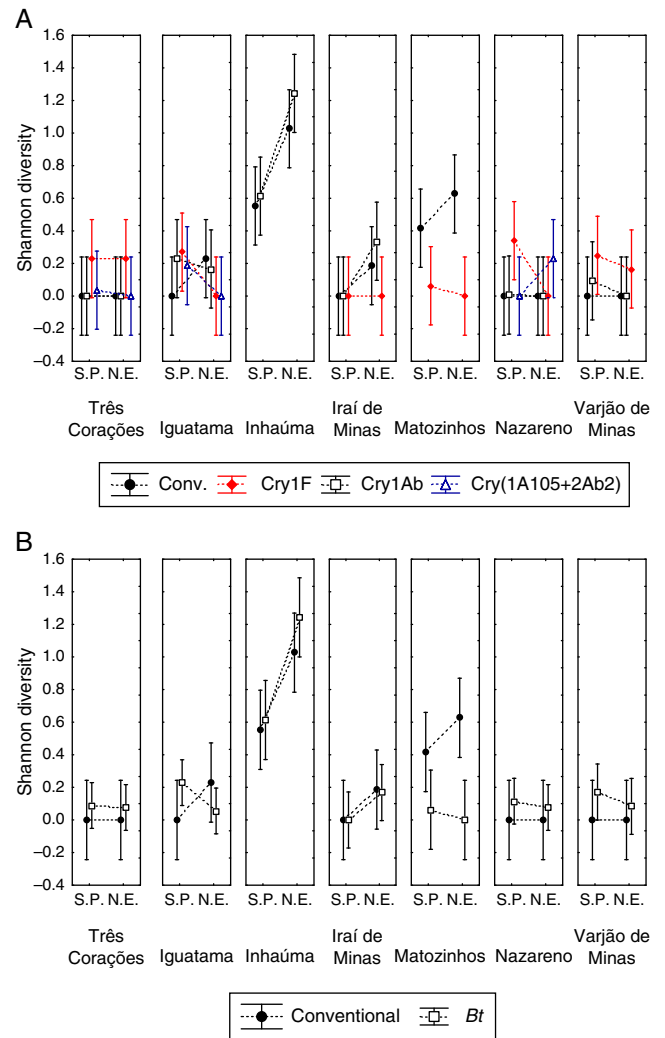


Fig. 15. Estimated diversity of secondary pests (S.P.) and natural enemies (N.E.) in tassels of conventional and transgenic maize for Cry1Ab, Cry1F and combined Cry2Ab2 and Cry1A105 proteins (A) and estimated richness in conventional maize and *Bt* maize (B), in different counties in Minas Gerais. Bars represent 95% confidence interval.

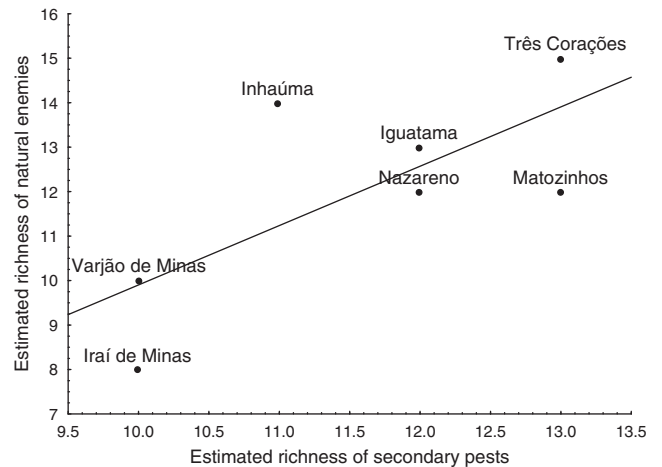


Fig. 16. Relationship between estimated richness of secondary pests and estimated richness of natural enemies in the studied cornfields.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

To Embrapa Milho e Sorgo and especially to the field and laboratory support team: Eustáquio Francisco Souza de Oliveira, Ismael M. Maciel and Ademilson S. da Rocha for their collaboration in this work.

References

- Bauer-Panskus, A., Then, C., 2014. Case study: industry influence in the risk assessment of genetically engineered maize 1507. *Testbiotech Background* 10, 1–32.
- Busato, G.R., Grützmacher, A.D., Garcia, M.S., Giolo, F.P., Zotti, M.J., Stefanello Jr., G.J., 2005. *Biologia comparada de populações de Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) em folhas de milho e arroz. *Neotrop. Entomol.* 34, 743–750.
- Busato, G.R., Grützmacher, A.D., Oliveira, A.C., Vieira, E.A., Zimmer, P.D., Kopp, M.M., Bandeira, J.M., Magalhães, T.R., 2004. Análise da estrutura e diversidade molecular de populações de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) associadas às culturas de milho e arroz no Rio Grande do Sul. *Neotrop. Entomol.* 33, 709–716.
- Capalbo, D.M.F., Dusi, A.N., Pires, C.S., Paula, D.P., Arantes, O.M.N., Melo, I.S., 2009. OGM e Biossegurança Ambiental. In: Costa, M.A.F., Costa, M.F.B. (Eds.), *Biossegurança de OGM: Uma visão integrada*. Publif, Rio de Janeiro, pp. 190–219, Available at: <http://www.fiocruz.br/ioc/media/101027-Biosseguranca%20de%20OGM.V1.pdf>.
- Carneiro, A.A., Guimarães, C.T., Valicente, F.H., Waquil, J.M., Vasconcelos, M.J.V., Carneiro, N.P., Mendes, S.M., 2009. Milho Bt: Teoria e prática da produção de plantas transgênicas resistentes a insetos-praga. *Embrapa Milho e Sorgo, Sete Lagoas (Circular Técnica 135)*.
- Colwell, R.K., 2000. Statistical estimation of species richness and shared species from samples (EstimateS) [6.0b1], Available at: <http://viceroy.eeb.uconn.edu/estimates> (accessed 15.04.13).
- Cruz, I., Silva, R.B., Figueiredo, M.L.C., Penedo-Dias, A.M., Del Sarto, M.C.L., Nuessly, G.S., 2011. Survey of ear flies (Diptera Ulidiidae) in maize (*Zea mays* L.) and a new record of *Euxesta mazorca* Steyskal in Brazil. *Rev. Bras. Entomol.* 55, 102–108.
- Dale, P.H., Clarke, B., Fontes, E.M.G., 2002. Potential for the environmental impact of transgenic crops. *Nat. Biotechnol.* 20, 567–574.
- Dively, G.P., 2005. Impact of transgenic VIP3A Cry1Ab Lepidopteran-resistant field corn on the nontarget arthropod community. *Environ. Entomol.* 34, 1267–1291.
- Fernandes, O.D., Parra, J.R.P., Neto, A.F., Pícoli, R., Borgatto, A.F., Demétrio, C.G.B., 2003. Efeito do milho geneticamente modificado mon810 sobre a lagarta-do-cartucho *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera: Noctuidae). *Rev. Bras. Milho Sorgo* 2 (2), 25–35.
- Fontes, E.M.G., Pires, C.S.S., Sujii, E.R., 2003. O impacto de plantas geneticamente modificadas resistentes a insetos sobre a biodiversidade. In: Pires, C.S.S., Fontes, E.M.G., Sujii, E.R. (Eds.), *Impacto ecológico de plantas geneticamente modificadas: o algodão resistente a insetos como estudo de caso*. Embrapa Recursos Genéticos e Biotecnologia, CNPq, Brasília, pp. 65–83.
- Frizzas, M.R., Oliveira, C.M., 2006. Plantas transgênicas resistentes a insetos e organismos não-alvo: Predadores, parasitóides e polinizadores. *Univ. Cienc. Saude* 4, 63–82.
- Heltshe, J.F., Forrester, N.E., 1983. Estimating species richness using the jackknife procedure. *Biometrics* 39, 1–11.
- Hilbeck, A., Baumgartner, M., Fried, P.M., Bigler, F., 1998. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environ. Entomol.* 27, 480–487.
- Krebs, C.J., 1999. *Ecological Methodology*, 2nd ed. Addison-Wesley Educational Publishers, Inc., Vancouver.
- Losey, J.E., Rayor, L.S., Carter, M.E., 1999. Transgenic pollen harms monarch larvae. *Nature* 399, 214.
- 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 Cry 1A(b). *Pesq. Agropec. Bras.* 46, 239–244.
- Meissle, M., Romeis, J., 2009. The web-building spider *Theridion impressum* (Araneae: Theridiidae) is not adversely affected by Bt maize resistant to corn rootworms. *Plant Biotechnol.* 7, 645–656.
- Meissle, M., Romeis, J., 2012. No accumulation of Bt protein in *Phylloneta impressa* (Araneae: Theridiidae) and other arthropods in Bt maize. *Environ. Entomol.* 41, 1037–1042.
- Naranjo, S.E., 2005. Long-term assessment of the effects of transgenic Bt cotton on the function of the natural enemy community. *Environ. Entomol.* 34, 1211–1223.
- O’Callaghan, M., Glare, T.R., Burgess, E.P.J., Malone, L.A., 2005. Effects of plants genetically modified for insect resistance on non-target organisms. *Annu. Rev. Entomol.* 50, 271–292.
- Paula, D.P., Andow, D.A., Timbó, R.V., Suiii, E.R., Pires, C.S.S., Fontes, E.M.G., 2014. Uptake and transfer of a Bt toxin by a Lepidoptera to its eggs and effects on its offspring. *PLoS One* 9, 1–7.
- Pilcher, C.D., Rice, M.E., Obrycki, J.J., 2005. Impact of transgenic *Bacillus thuringiensis* corn and crop phenology on five nontarget arthropods. *Environ. Entomol.* 34, 1302–1316.
- Pires, C.S.S., Sujii, E.R., Fontes, E.M.G., 2003. Avaliação ecológica de risco de plantas geneticamente modificadas resistentes a insetos sobre inimigos naturais. In: Pires, C.S.S., Fontes, E.M.G., Sugii, E.R. (Eds.), *Impacto ecológico de plantas geneticamente modificadas: o algodão resistente a insetos como estudo de caso*. Embrapa Recursos Genéticos e Biotecnologia, Brasília, pp. 85–115.
- Raybould, A., Stacey, D., Vlachos, D., Graser, G., Li, X., Joseph, R., 2007. Non-target organisms risk assessment of MIR604 maize expressing mCry3A for control of corn rootworms. *J. Appl. Entomol.* 131, 391–399.
- Romeis, J., Meissle, M., Raybould, A., Hellmich, R.L., 2009. Impact of insect-resistant transgenic crops on above-ground non-target arthropods. In: Ferry, N., Gatehouse, A.M.R. (Eds.), *Environmental Impact of Genetically Modified Crops*. CAB International, Wallingford, pp. 165–198.
- Romeis, J., Meissle, M., Naranjo, S.E., Li, Y., Bigler, F., 2014. The end of a myth – Bt (Cry1Ab) maize does not harm green lacewings. *Front Plant Sci* 5, 1–10.
- Schuler, T.H., Potting, R.P.J., Denholm, I., Poppy, G.M., 1999. Parasitoid behavior and Bt plants. *Nature* 400, 825–826.
- Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattila, H.R., Siegfried, B.D., Dively, G.P., 2001. Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11937–11942.
- Vasconcelos, M.J.V., Carneiro, A.A., Valicente, F.H., 2011. Estudo de caso em milho Bt. In: Borém, A., Almeida, G.D. (Eds.), *Plantas geneticamente modificadas: desafios e oportunidades para regiões tropicais*. Universidade Federal de Viçosa, Viçosa, pp. 311–332.
- Waquil, J.M., 1997. Amostragem e abundância de cigarrinhas e danos de *Dalbulus maidis* (De Long & Wolcott) (Homoptera: Cicadellidae) em plântulas de milho. *An. Soc. Entomol. Bras.* 26, 27–33.
- Waquil, J.M., Vitousek, P.M., Siegfried, B.D., Foster, G.N., 2004. Atividade biológica das toxinas do Bt, Cry1A(b) e Cry 1F em *Spodoptera frugiperda* (SMITH) (Lepidoptera: Noctuidae). *Rev. Bras. Milho Sorgo* 3, 153–163.
- White, J.A., Andow, D.A., 2005. Host-parasitoid interactions in a transgenic landscape: spatial proximity effects of host density. *Environ. Entomol.* 34, 1493–1500.