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Biological Control and Crop Protection

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

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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.

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).

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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 cropsRegarding 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	*Bt* 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