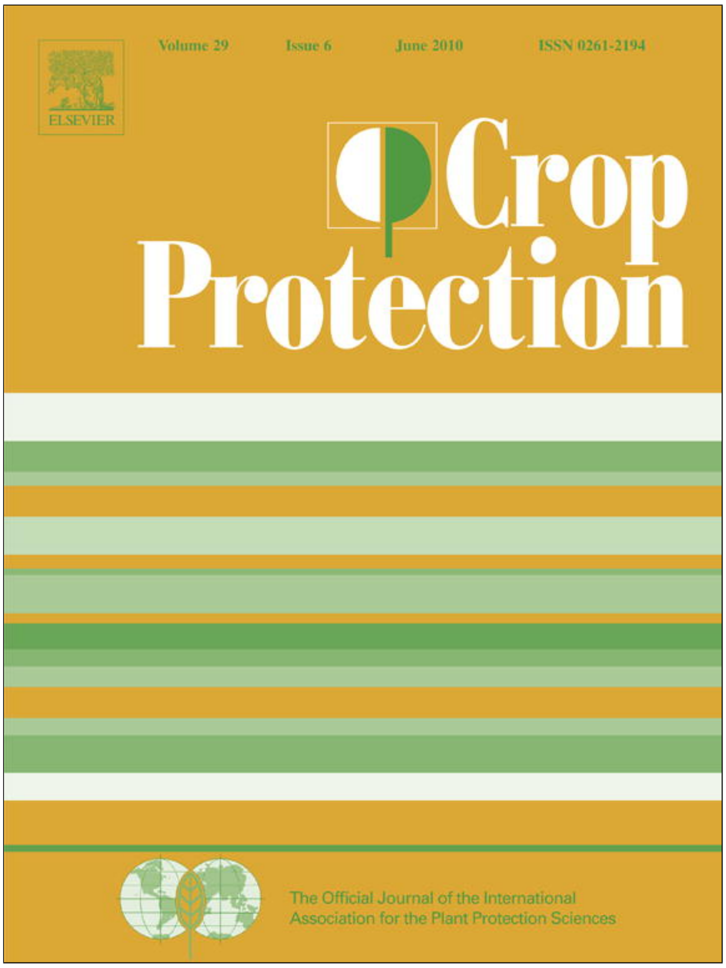
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Short communication

A preliminary study on the effects of a transgenic corn event on the non-target pest *Dalbulus maidis* (Hemiptera: Cicadellidae)

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ABSTRACT

The inclusion of the *cry* gene in corn may produce direct effects on non-target pests. Our research was focused on the relationship between Bt corn germplasm, expressing the *cry*1F protein to control the fall armyworm [*Spodoptera frugiperda* (Noctuidae)], and a non-target pest, the corn leafhopper [*Dalbulus maidis* (Cicadellidae)]. The aim of this contribution was to elucidate if Bt corn plants have influence on the oviposition preference of the leafhopper and to evaluate the effect of the transgenic plant on the hatching rate of egg. Female corn leafhoppers were released in cages each containing two potted plants in the V2 stage: a Bt germplasm and the corresponding isogenic hybrid. Laid eggs were counted and the number of hatched nymphs recorded. *D. maidis* females ovipositted and laid more eggs in Bt plants. The egg hatching rate was negatively affected by the Bt germplasm. In addition, a field study was conducted in order to determine the abundance of *D. maidis* adults in Bt corn and the corresponding non-Bt isoline. Two corn plots sown with the same germplasms as used in the laboratory bioassays were sampled weekly. In the field, the population of the corn leafhopper was higher in the Bt corn plot than in the non-Bt isoline. Possible hypotheses for the differences in abundance of the vector in the field are: a) that pleiotropic effects of Bt corn could attract adults; b) the existence of a possible direct competition between the corn leafhopper and the target pest in order to utilize the whorls of corn plants as refuge and feeding sites, so the high populations of the vector could be due to the large supply of healthy whorls in the transgenic plot; and/or c) a differential attack of natural enemies occurring in non-Bt plots.

1. Introduction

In developing countries, there exists not only a lack of understanding as to how effective engineered crops would be in providing protection against target pests, but also a lack of information on the pests of almost all the important crops, and the effects of introduction of transgenic crops on the population dynamics of target and non-target insects have not been addressed yet.

The *Bacillus thuringiensis* (Bt) δ-endotoxin gene has been incorporated into corn DNA so that the corn produces a crystal-like protein (*cry*) that is toxic mainly to Lepidoptera once ingested. Bt-transgenic corn was commercially introduced in Argentina in 1996, and at present there are four different Bt corn events in use (CONABIA, 2009). The total area planted with Bt corn in 2008/09 was ca. 2.34 million ha, about 73% of the total area planted with corn in Argentina (ARGENBIO, 2009). Some events express *cry* proteins especially noxious to the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). The FAW is a polyphagous species that causes important damage to diverse crops in different regions of America (Sparks, 1979), and it is the most important economic pest affecting corn crops in Argentina (Virla et al., 1999).

There is growing concern that genetically engineered crops may pose risks to natural and agricultural ecosystems (Groot and Dicke, 2002). The transformation of corn with the *cry* gene may lead to pleiotropic effects that could have consequences for non-target pest behavior and/or give rise to other ecological implications (Conway, 2000). In addition, certain authors have stated that with the deployment of transgenic crops secondary pests will no longer be controllable in the absence of sprays for major pests, so they will become new potential key pests (Sharma and Ortiz, 2000).

Transgenic plants that express Bt endotoxins are effective for the control of insects like Lepidoptera and Coleoptera that feed on them by chewing or biting. However, there is little evidence about the potential effects of such plants against sap sucking pests (Maqbool et al., 2001),

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like the corn leafhopper, *Dalbulus maidis* (DeLong and Wolcott) (Hemiptera: Cicadellidae). Sucking insects do not feed on plant cells but on sap, which does not contain Bt toxins (Raps et al., 2001).

The corn leafhopper is a very important corn pest in Latin America because it is an efficient vector of several plant pathogens: corn stunt spiroplasma (CSS, *Spiroplasma kunkelii* Whitcom et al.), maize bushy stunt phytoplasma and maize rayado fino marafivirus (Nault, 1990; Nault and Ammar, 1989). The diseases caused by these pathogens adversely affect the corn crop; CSS is the most important pathogen in Argentina due to its high incidence in subtropical areas (Giménez Pecci et al., 2002; Virla et al., 2004). *D. maidis* shows a broad distribution throughout the Americas: it has been detected from southeastern and southwestern USA to Argentina (Triplehorn and Nault, 1985). In Argentina, it is the most common leafhopper feeding on corn (Luft Albarracin et al., 2008).

As Argentina's economy largely depends on agricultural activities and both FAW and CSS are very important adverse factors of corn-crop productivity we focused our research on the relationship between the relatively recently introduced Bt corn germplasm and its impact on the CSS vector, a non-target pest. The goal of this study was to address the following two questions: 1) Is there a preference of female *D. maidis* for oviposition in Bt corn plants? and 2) Do Bt corn plants affect the egg hatching rate? To confirm the interpretation of preference data from the laboratory, a field survey was conducted in order to determine if *D. maidis* adults inhabit Bt corn and the isogenic variety without *cry* gene to an equal degree.

2. Materials and methods

In order to assess the oviposition preference, a female (aged 6–10 days) and a male leafhopper were released in a cage entirely covered with a cotton mesh (50 × 50 × 50 cm) that contained two potted plants [vegetative stage, with two visible leaf collars (V2)]: one with a Herculex® Elite germplasm, expressing the *Cry*1F gene, and the other with the non-Bt 30F87 isoline hybrid. The assay consisted of 70 replicates carried out in a greenhouse at 24 ± 4 °C and 60–80% RH with natural light. Corn seedlings were grown in 0.8 L pots, which did not receive any fertilization treatments, and were maintained outdoor in anti-aphid cages. The position of the Bt and isogenic plants in the cages were alternatively changed in the different replicates. Plants were exposed to oviposition for 48 h and then the leafhoppers were removed. After eight days, the leaves containing eggs were cut off the plants and transferred to Petri dishes, which contained wet tissue paper on the bottom. Dishes were covered with polyethylene film to avoid desiccation of eggs and leaves and to prevent any emerging nymphs from escaping. Laid eggs were counted under a binocular microscope and the number of hatched nymphs was recorded. After 5–7 days the eggs became easier to see as they turned white and had red eyespots. These endophytic eggs are typically deposited in the mid-veins of leaves of young corn plants, generally in the whorl. Occasionally, several eggs would be oviposited in a row.

The field study was carried out during the 2009 growth season (sowing date 21 January 2009) in two corn plots each of 0.25 ha. One plot was sown with Bt Herculex® seeds and the other with the isogenic 30F87 (the same germplasms as used in the laboratory bioassays). The plant density was 57,000 plants per ha. Rows were spaced 0.52 m apart. The crop was surrounded by wild vegetation. The plots were located in Las Breñas (Chaco province; 27°05′20″S, 61°06′20″W, elevation: 102 m). They did not receive any pesticide or fertilization treatment. Samples of *D. maidis* were taken weekly, from V2 to V8 phenological stages. During each sampling date, 10 groups of 10 plants each were randomly chosen and searched for corn leafhoppers resting or feeding on the corn plants: individual plants were thoroughly examined for adult insects. During the first phenological stages, leafhopper recordings were carried out by covering the plants with polyethylene bags, and then insects were collected using manual vacuum aspirators according to the technique by Cuadra and Maes (1990). After the identification using magnifier lens, the specimens were returned to the field. All samples were taken in the morning, between 9:00 and 12:00 a.m.

The preference of females to oviposit in the offered germplasms was analyzed using a transformation of "Ivlev's electivity index" (E) and the "Ivlev's forage ratio" (E′) (Ivlev, 1961). Ivlev compared the relative accessibility of food types in the environment (p) and their relative utilization in the diet (r) as follows:

$$E_{(i)} = \left(r_{(i)} - p_{(i)}\right) / \left(r_{(i)} + p_{(i)}\right) \text{ and } E'_{(i)} = r_{(i)}/p_{(i)}$$

In our case, however, $p_{(i)}$ was considered the relative availability of the germplasms "i" and $r_{(i)}$ the relative utilization of the "i" plants for oviposition in the experiment. Additionally, the data were analyzed by means of the Fisher's exact test, considering the total number of oviposited and non-oviposited plants.

A paired Mann Whitney test (two-tailed) was used for comparison of the number of deposited eggs and differences in the percentage of egg hatching were analyzed using the Mann Whitney test (one-tailed). Field differences in *D. maidis* abundance between the treatments were analyzed with a t test. The normality of the data was checked using the D'Agostino and Pearson omnibus normally tests. Statistical analysis was performed using GraphPad Prism (version 5.00) with a significance level of $P = 0.05$.

3. Results

3.1. Laboratory studies

From the 70 pairs of plants exposed in the laboratory, 49 received eggs in both germplasms, 17 only in one germplasm (12 Bt and 5 isogenic) and four pairs did not receive any eggs: transgenic plants were more oviposited (87.1%) than isogenic ones (77.1%). The electivity index showed a slight preference for oviposition in transgenic germplasm ($E_{(bt)} = 0.27$ and $E_{(non\text{-}Bt)} = 0.21$), and similar for the "oviposition ratio" ($E'_{(bt)} = 1.74$ and $E'_{(non\text{-}Bt)} = 1.54$). However, the Fisher's exact test did not reveal any significant differences between the two treatments ($P = 0.1304$).

More eggs were deposited on transgenic plants (438) than on isogenic ones (311) with a mean of 6.3 and 4.4 eggs/plant, respectively, but the difference was not statistically significant (Mann Whitney test; $P = 0.0563$).

The percentage of eggs that hatched was 6.6% lower in transgenic than in isogenic plants; 381 nymphs emerged from 61 oviposited Bt plants (87.0%) and 291 from 54 isogenic plants (93.6%). Statistical analysis of the data showed significant differences (Mann Whitney test; P: 0.0093): the percentage of emerging nymphs was higher when eggs were deposited in non-Bt plants.

3.2. Field study

In the field study, the number of adult corn leafhoppers collected during the entire study was low (Table 1). Vector population in both plots was similar in the earlier stages of the crops, but later there was an increase in the abundance in the transgenic plot while vectors were absent in the isogenic plants, a difference which was statistically significant (t test; $P < 0.0001$).

4. Discussion and conclusions

D. maidis females oviposited and laid more eggs in Bt plants, but the difference was not significant to affirm a clear tendency to

Table 1
Abundance of *D. maidis* in two plots sown with a Bt corn and the corresponding isogenic in Las Breñas (Chaco province, Argentina), and percentage of plants showing damages in the whorls by fall armyworm larvae.

Phenological stage[a]			V_2	V_4	V_5	V_6	V_8	Total
Vector abundance (adults/plant)	Bt corn	n[b]	1	8	7	2	7	25
		mean[c] (SE)[d]	0.01 a (0.010)	0.08 a (0.031)	0.07 a (0.026)	0.02 a (0.014)	0.07 a (0.026)	0.05 a (0.0107)
	Non-Bt corn	n	1	0	0	0	0	1
		mean (SE)	0.01a (0.010)	0 b (−)	0 b (−)	0 a (−)	0 b (−)	0.002 b (0.0001)
% FAW affected whorls[e]	Bt corn		0 A	4 A	10 A	0 A	0 A	2.8 A
	Non-Bt corn		21 B	70 B	59 B	42 B	92 B	56.8 B

[a] "V_n" plants in vegetative stage, where "n" is the number of visible leaf collars.
[b] Total number of *D. maidis* recorded in the samples.
[c] Vector abundances followed by the same letter within columns for the Bt corn or non-Bt corn do not differ significantly according to t test at $P \geq 0.05$.
[d] SE = standard error.
[e] Percentage of affected whorls followed by different letters within columns for the Bt corn or non-Bt corn differs significantly according to t test at $P \leq 0.05$.

oviposition in transgenic germplasms. So far, only the attractancy and oviposition preference for the corn leafhopper to different non-transgenic corns were determined, but no obvious differences were found (Collins and Pitre, 1969). It is important to mention that our assays were carried out with V2 plants; if the study had been conducted with more developed plants, leafhoppers could have shown a greater difference in preference. Oviposition preference of Cicadellidae was strongly influenced by vein characteristics of the leaves, plant chemistry and presence of foliar pubescence among other features (Alyokhin et al., 2004; Irvin and Hoodle, 2004). There is ample evidence of variations within insect populations with regard to the choice of host plants for oviposition and feeding, and this phenotypic variation has a genetic component; alternative and novel hosts are acceptable to some individuals and there is a potential for rapid host shifts (Sezer and Butlin, 1998).

The egg hatching rate was affected in the Bt germplasm. The vector eggs are laid endophytically and to emerge, the nymphs must to force the leaf tissue (cuticle, and partly mesophyll) over the chorion. Saxena and Stotzky (2001) demonstrated through the study of eight events that Bt corn had higher lignin content than non-bt corn (33–97% higher). Lignin is a major structural component of plant cells that confers strength, rigidity, and impermeability to water. Pleiotropic effects in the used Bt corn probably hindered the nymphs from emerging normally. Future studies will have to be carried out to clarify the reasons for this phenomenon.

In the field study, populations of the corn leafhopper were higher in the Bt corn plot than in the non-Bt isoline. Although the reasons for colonization of corn crops expressing *cry* toxins by female *D. maidis* were not examined, according to the results obtained in our laboratory, the noticeable increase in adults in the Bt corn plot would not be a result of female oviposition preference at an early stage of crop development. Several hypotheses for the marked differences in abundance of the vector in the field have been formulated, and at present we are trying to answer them:

a) Considering that any modification in components of plant cells could have ecological implications, pleiotropic effects in the Bt corn here studied could attract adults. This would mean that the transgenic plants possibly have chemical (nitrogen, sulphur, lignin, terpenoids, chlorophyll, surface waxes, etc.), morphological (solidity of leaves, presence and density of trichomes, etc.) and/or physical (color) characteristics that affect *D. maidis*' landing behavior on and/or permanency in the crop. Differences between diverse chemical or morphological characteristics in Bt-transgenic plants and its corresponding non-Bt near-isolines are currently under study [for references see Saxena and Stotzky (2001) and Subedi and Ma (2007)]. Schetino Bastos et al. (2007) demonstrated that attacks by *D. maidis* were more common in corn plants with higher contents of nitrogen, sulphur, calcium and copper in the leaves.
b) There exists a direct competition between the corn leafhopper and the FAW regarding the utilization of whorls of corn plants as refuge and feeding sites and consequently, the high populations of the corn leafhopper observed in the field study could be due to a large supply of healthy whorls in the transgenic plot. Badji et al. (2004) found significant correlations between FAW and *D. maidis* populations in corn crops in Brazil and suggested possible overlooked interactions.
c) A less plausible hypothesis would be a differential attack rate by natural enemies occurring in non-Bt plots. If this occurred, considering that the differences in vector abundance were recorded for adults, it could be due to the presence of generalist predators. Taking into consideration the life cycle of the vector and its known parasitoids, the action of the latter ones could not be the reason for the dissimilar levels reached during the sampled period.

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