# Genetically modified (GM) corn in the Philippines : Ecological impacts on agroecosystems, effects on the economic status and farmers' experiences

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# Asian Corn Borer (ACB) and non-ACB pests in GM corn (Zea mays L.) in the Philippines

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## Abstract

The Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée), has become the most damaging pest in corn in south-east Asia. Corn farmers in the Philippines have incurred great yield losses in the past decades because of ACB infestation. *Bacillus thuringiensis* (*Bt*) and *Bt* herbicide-tolerant (*Bt*HT) corns have been developed to reduce borer attacks worldwide. This study assessed the extent of ACB and non-ACB pest infestations in both GM and non-GM corn in Isabela Province, the Philippines. Specific aims were to reinvestigate the efficacy of *Bt* corn in controlling ACB, to evaluate what parts of *Bt* corn plants are susceptible to ACB, to monitor the potential development of ACB resistance and to evaluate whether secondary pests dominate in an ACB-free *Bt* corn environment. The study involved preparatory interviews with farmers, site selection, field scouting and visual inspection of 200 plants along 200m transect lines through 198 cornfields.

*Bt* corn can efficiently reduce the ACB pest problem and reduce borer damage by 44%, to damage levels in *Bt* and *Bt*HT corn of 6.8 and 7%, respectively. The leaves of *Bt* corn were more susceptible, while cobs of *Bt* corn were less affected by ACB. Non-ACB pests were common in *Bt* toxin-free cornfields and reduced in non-GM cornfields where ACB was abundant. No secondary pest outbreaks were found in ACB-free *Bt* cornfields.

*Bt* and *Bt*HT corn hybrids containing the Cry1Ab protein performed well in Isabela Province. Reduced cob damage by ACB on *Bt* fields could mean smaller economic losses even with ACB infestation. The occurrence of ACB in *Bt* and *Bt*HT cornfields, although at a moderate and insignificant level, could imply the potential development of resistance to *Bt* toxin.

## Introduction

The damage brought about by corn borer infestation constitutes a major constraint on agriculture in all of the world's corn-producing countries. According to the Food and Fertiliser Technology Centre (FFTC), even minor damage can lead to a low market value of corn. In China, the Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée), is an important component of the lepidopteran pest complex for cotton and corn (He *et al*., 2003; He *et al*., 2006). In the Philippines, ACB has become the most damaging corn pest (Logroño, 1998). Tropical agriculture countries such as the Philippines are threatened by a high incidence of ACB, with a 27% corn yield reduction resulting from 40–60% corn borer infestation (Logroño, 1998). The rate of infestation can be as high as 80% when corn is planted late (Javier, 2004).

Most farmers prefer a readily available, labour-reducing and easy method of ACB control using insect-resistant (*Bt* and *Bt*HT) corn. *Bt* and *Bt*HT corns have a genetically built-in endotoxin Cry1Ab protein from the *Bacillus thuringiensis* (*Bt*) bacterium, which kills ACB as activated toxin binds to the midgut of the corn borer, leading to ion influx, cell lysis and the death of the susceptible organism (Schnepf *et al*., 1998; Hua *et al.,* 2001). *Bt*-protected corn reduces the damage rates by ACB (He *et al.,* 2006). In temperate countries, the use of *Bt* corn has become an effective tool to control European corn borer (ECB), *Ostrinia nubialis* Hb (Rice and Pilcher, 1997). Transgenic corn proved to be effective against ACB, reducing borer tunnels by 99% and leaf injury by 84% (Thomson *et al.,* 2010), and affording a yield of 9838 kg $ha^{-1}$ (Dekalb 818 YG) compared with 7838 kg $ha^{-1}$ with conventional corn (NK 8870) (Philippine NSIC, 2011).

Herbicide-tolerant corn (HT and *Bt*HT) is genetically modified to counteract the damaging effects of herbicides. Specifically, HT corn is protected from glyphosate through its genetically built in EPSP (5-enolpyruvylshikimate-3-phosphate synthase) cDNA, isolated from a glyphosate-tolerant petunia cell culture line. (Padget *et al.,* 1995). Cultivation of HT corn can lead to substantial environmental benefits (Cerdeira and Duke, 2006), as it allows minimal or zero tillage methods. A new investigation of ACB plant damage in the Philippines was carried out to ensure that endotoxin-containing *Bt* corn still exhibits the same efficacy in ACB control as in previous surveys. In the 2003-2004 growing season, Isabela farmers reported no borer damage in *Bt* corn, compared with 15% crop damage in non-*Bt* corn. In 2007-2008, non-GM corn farmers in Isabela encountered a low percentage of damage by ACB (4%), while *Bt* corn farmers reported zero damage (Gonzales *et al*., 2009).

The present study also included a comparative assessment between leaves, stems and cobs of *Bt* corn plants, to test whether plant parts exhibit different resistance to ACB, as the Cry1Ab protein that produces the *Bt* endotoxin is known to occur in different concentrations in different plant parts (Abel and Adamczyk, 2004; Székács *et al*., 2010a; Székács *et al*., 2010b). Previous studies (Halpin *et al*., 1994; Saxena & Stotzky, 2001) showed that *Bt* toxin is not the only feature specific to *Bt* corn. *Bt* corn also has a 33-97% higher lignin content than non-*Bt* corn (Saxena and Stotzky, 2001; Poerschmann *et al*., 2005). Lignin in a plant confers strength, rigidity and water impermeability, which contributes to its protection from borers and is effective against second-generation ECB (Ostrander and Coors, 1997). Differences in lignin content between plant parts could imply differences in resistance against and/or susceptibility to corn borer infestation.

This study also evaluated the recent actual field situation regarding the potential development of ACB resistance to *Bt* corn in the Philippines. In China, both field and laboratory (He *et al*., 2003; Wang *et al*., 2004; Chang *et al*., 2007) have been carried out to evaluate the resistance of *Bt*11 and Mon810 to ACB. Until 2009, only field surveys of ACB host plants other than corn were carried out in the Philippines (Caasi-Lit *et al*., 2004; Caasi-Lit *et al.,* 2009). Recently, Alcantara *et al*. (2011) provided direct evidence from laboratory bioassays that ACB populations in the Philippines have remained susceptible to *Bt* corn. Outside the laboratory, however, many factors should be taken into account, as the amounts ingested by the ACB, the mode of exposure and the sources of *Bt* toxin vary between plant parts, field situations and locations.

As reported in Gerpacio *et al*. (2004), other non-ACB corn pests in the Philippines, such as armyworm (*Pseudaletia unipunctata*) and common cutworm (*Spodoptera litura* Fabricius), can occur in GM corn and cause moderate to high yield losses. An evaluation was therefore made to establish whether widespread adoption of *Bt* and *Bt*HT corn could potentially cause outbreaks of secondary pests (non-ACB) in time. If these corn hybrids efficiently eradicated ACB, non-ACB pests could establish populations and encroach on the corn environment, where ACB is nearly or completely absent. Earlier studies showed that *Bt* corn can partially control and reduce the damage of other, non-ACB, pests such as corn earworm (*Helicoverpa zeae*), common stalk borer (*Papiapema nebris*) and armyworm (Pilcher *et al*., 1997; Lynch *et al.,* 1999). To verify this in a tropical environment, an investigation was made not only of the damage caused by ACB but also of plant damage by pests other than ACB.

To the present authors' knowledge, there has been no recent field research into ACB infestation since the emergence of new *Bt* corn hybrids. In particular, there is a lack of empirical studies in actual field situations assessing *Bt* and *Bt*HT performance and the susceptibility of ACB in the Philippines. Hence, the present study aimed to: (1) investigate once more the performance of widely cultivated GM corn in terms of ACB control; (2) determine the susceptibility of different plant parts containing *Bt* endotoxin to borer attacks; (3) assess the potential development of ACB resistance in a GM corn environment; (4) evaluate the potential build-up of secondary pests in an ACB-free GM corn environment.

Lastly, as environmental factors may affect the occurrence of ACB or non-ACB pests, and the spatial distribution of these factors is unknown, spatial variables (i.e. distance to the river, geographical location and elevation) of the cornfields were taken into consideration in the analyses to ensure that location factors did not bias the results.

## Methods

*Description of the study area*

Isabela Province is situated in the north-east Luzon part of the Philippines (Fig. 1). It has a type III climate with an average rainfall of 1700 mm and an annual temperature of 27 $^{0}$C (Gerpacio *et al.*, 2004). Clay loam and sandy loam are the most common soil types in the fields surveyed. Corn production in the province starts at the first monsoon rains, during April or May. The first cropping ends in August and most non-GM farmers start planting for the second crop immediately after harvest. By contrast, most GM corn farmers wait until November or December to start planting for the second cropping. This is because Isabela is a typhoon- and flood-prone area, and 90% of GM corn farmers whose fields are located in lowlying areas do not want to take the risk. The province is transected by the Cagayan River, and most of the cornfields suitable for corn production are located near this river. The distance from the cornfields surveyed to the Cagayan River ranges from 0.8 to 32 km. Elevation also differs between cornfields, ranging from 0.04 to 0.15 km above sea level. The size of the inspected cornfields ranges from 0.5 to 4 ha.

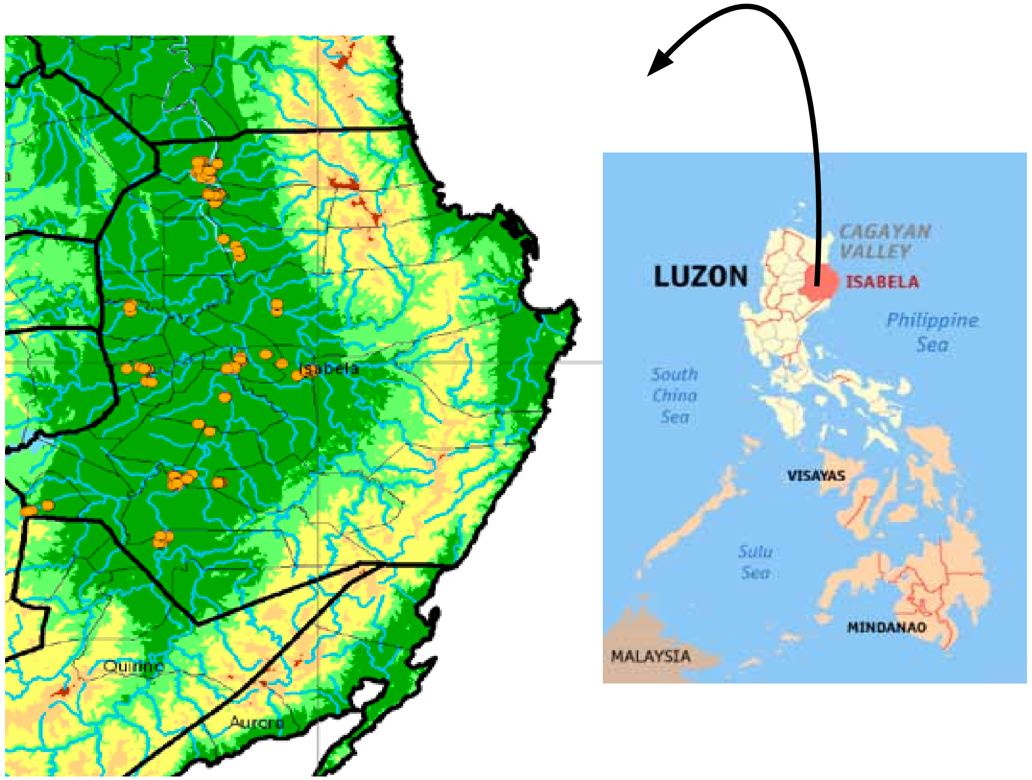


**Figure 1.** Map showing locations of 198 surveyed cornfields in 19 municipalities of Isabela province, in the Northeast Luzon part of the Philippines, during the 2010 wet growing season.

*Selection of cornfields*

As there are three GM corn hybrids (*Bt*, *Bt*HT and HT) that are widely cultivated in the Philippines, all three were included in the study. Non-GM corn was used for the comparison. The starting points for selecting field sites were villages with a high degree of adoption of GM corn and a history of serious ACB infestation. The authors interviewed village officials and/or municipal agricultural officers to find out the locations of corn areas. Prior to field inspections, interviews were held with farmers to ascertain the specific corn type planted in the fields. Permission to conduct visual inspections was obtained from the owners or current tillers of the fields. Forty-nine villages in 19 municipalities of Isabela were surveyed (Fig. 1). In all, 198 cornfields were individually inspected, in the post-flowering to mid-maturity stages, during the wet growing season of 2010 (Table 1).

**Table 1.** Numbers of GM and non-GM cornfields visited during the survey of 198 fields in Isabela province, The Philippines, in the 2010 wet growing season.

| Municipality (n=19) | Number of Fields (n=198) | *Bt* (n=30) | *Bt*HT (n=91) | HT (n=14) | non-GM (n=63) |
|---|---|---|---|---|---|
| Angadanan | 12 | 1 | 5 | 0 | 6 |
| Aurora | 10 | 1 | 8 | 0 | 1 |
| Benito Soliven | 4 | 1 | 3 | 0 | 0 |
| Cabagan | 19 | 6 | 6 | 2 | 5 |
| Cabatuan | 8 | 0 | 5 | 0 | 3 |
| Cordon | 9 | 1 | 3 | 3 | 2 |
| Cauayan | 9 | 2 | 5 | 0 | 2 |
| Echague | 19 | 5 | 10 | 0 | 4 |
| Ilagan | 7 | 3 | 3 | 0 | 1 |
| Jones | 10 | 0 | 7 | 0 | 3 |
| Naguillan | 8 | 0 | 7 | 0 | 1 |
| Reina Mercedes | 10 | 1 | 1 | 0 | 8 |
| Roxas | 5 | 0 | 3 | 1 | 1 |
| San Guillermo | 15 | 0 | 7 | 2 | 6 |
| San Mariano | 8 | 1 | 4 | 1 | 2 |
| San Pablo | 14 | 6 | 0 | 1 | 7 |
| Sta. Maria | 7 | 1 | 3 | 1 | 2 |
| Sto. Tomas | 14 | 0 | 7 | 1 | 6 |
| Tumauini | 10 | 1 | 4 | 2 | 3 |

*Assessment of infestation*

The authors re-investigated the performance of GM corn in terms of the control of ACB by means of field scouting (Fishel *et al*., 2001) accomplished by determining the number of plants damaged in GM and non-GM corn. A 200 m transect line was established through the middle of each surveyed cornfield. Along this line, 200 plants were examined for probable signs of ACB infestation. ACB is a flat, scale-like, whitish lepidopteran that lays 25-50 eggs per egg mass (Gonzales *et al.*, 2009). Among the characteristic types of damage caused by ACB (Morallo and Punzalan, 2001) were: pinholes in leaves; big holes in stalks, the base of tassels or ear shanks; broken stalks and tassels; clumping of tassels; partial destruction of cobs; dropping of ears in severe cases (Fig. 2a). Damage by pests other than ACB (Fig. 2b) was recorded as the presence of injury damage characteristic of each specific pest.

The second objective was addressed by comparing the damage between plant parts by counting and recording the ACB-associated numbers of holes in leaves, cobs and stems. In addition, numbers of egg masses per plant were recorded to assess the behaviour of adult ACB on GM corn. The third objective was addressed by recording the extent of plant damage from ACB in all *Bt* and *Bt*HT cornfields. The percentage of damage per corn type was assessed, and the data produced in this study were compared with the findings of previous surveys in the Philippines, as reported by Gerpacio *et al*. (2004).

The final objective of this study, to assess the development of secondary pests, was addressed by counting the numbers of plants damaged by non-ACB pests. Among the non-ACB pests (Fig. 2b) observed during field inspections were aphids, black cutworm, armyworm, black crickets, grasshoppers, corn earworm, rootworm, termites and large pests such as birds and rats. Injury caused by black crickets and grasshoppers was indicated by the corrugated appearance of affected eaten leaf parts, going from the leaf margin towards the leaf midrib. Armyworm larvae cause irregular leaf holes with a maximum diameter of 0.6 cm, depending on the age and body size of the larvae. Damage by termites was very clearly identifiable, because of the evidence of plant injury created by soil line along the stem. Corn earworm injury was seen in the cobs as holes and feeding damage on the tips and soft parts of the husks and grains.

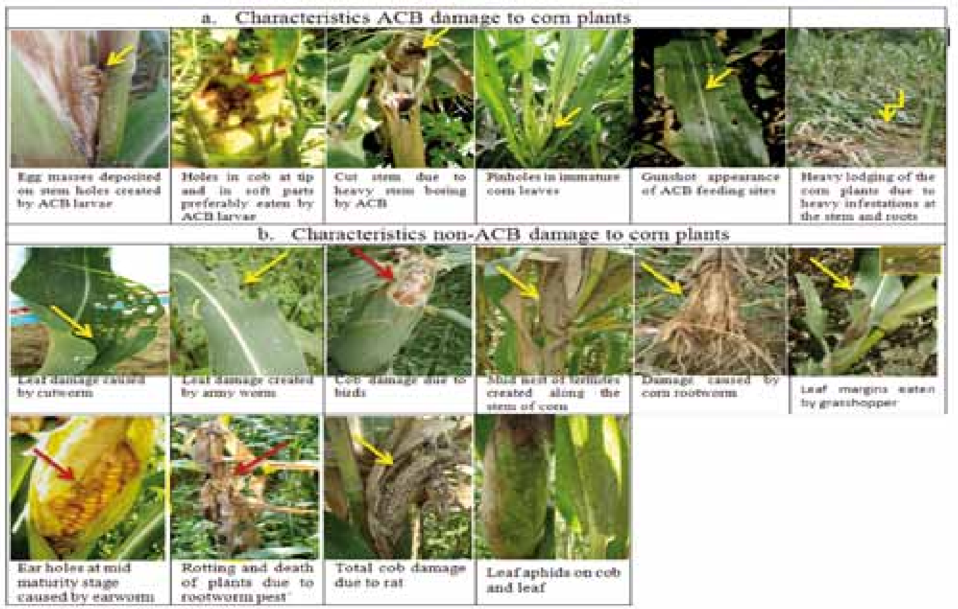


**Figure 2.** Observable damage symptoms of ACB (a) and non-ACB (b) infestations in corn plants surveyed in Isabela province, The Philippines, during the 2010 wet growing season.



*Spatial variables*
The authors tried to evaluate whether the present findings could be biased by spatial variables. Spatial data such as distance to the river, elevation and absolute and relative location of cornfields were gathered and incorporated in the statistical model. The distance of each field to the river was computed after obtaining the individual GPS (geographical position system, Garmin Vista eTrex) readings, and calculated in a GIS (geographic information system) using ArcGIS 9.2 software and the Hawth Tools extension. The relative locations of the cornfields were classified as southern, northern and central parts. As Ilagan is the capital of the province and centrally located (going from north to south), it was used as the georeference for the cornfield locations (Fig. 1). All cornfields belonging to towns to the north and south of the central town of Ilagan were categorised as northern and southern relative locations respectively. Absolute location is the actual location of the cornfield in GPS coordinates (*Y*-coordinates/longitude and *X*-coordinates/latitude).



*Statistical analysis*
Correlations between independent variables were analysed to identify the links between variables. Variables with low correlation values were selected for multivariate analyses. All data indicated the number of damaged plants per field (out of the 200 plants examined). Non-normally distributed data, assessed on the basis of residual plots (residual versus fitted, normal QQ, scale location and residual versus leverage) were ln($x$+1) transformed.

Multiple regression analysis was carried out per response variable, i.e. ACB damage, non-ACB damage or overall damage. The independent variables that were included in the statistical model were corn types (*Bt*, *Bt*HT, HT and non-GM), relative location of the cornfield (southern, central or northern), absolute location (latitude and longitude), distance to the river and elevation.

All analyses were done using R statistics v.2.12.2. The proportional test in R was used to compare damage to different plant parts between *Bt* (*Bt* and *Bt*HT) and non-*Bt* (non-GM and HT) corn. Specifically, this two-sample test for equality of proportions was employed to determine the number of *Bt* and non-*Bt* plants showing ACB holes in leaves, stems and cobs, as well as the number of plants with egg masses. Only significant results are presented in Sections 3 and 4, unless otherwise indicated.

**Results**

*Effects of GM corn on pest damage*
The percentages of plants with damage associated with ACB and non-ACB pests are given in Table 2. One field had a very high number of plants damaged by non-ACB pests. This made the present data non-normally distributed, making it necessary to transform the data using ln($x$+1). All the other values were natural log transformed. Corn type affected the amount of damage by ACB (Table 3, Fig. 3). Non-GM corn had the highest proportion of damaged plants, followed in descending order by HT, *Bt* and *Bt*HT corn (Table 3). *Bt* toxin in corn effectively reduced ACB

damage by 44%, from 15.7% in non-GM to 6.8% in *Bt* corn (Table 2). Non-ACB plant damage did not differ between GM and non-GM corn types (Table 3, Fig. 4). Among GM corn varieties, *Bt* corn had the highest number of non-ACB plant damage (Fig. 4). On overall damage (i.e. plant damage caused either by ACB or non-ACB), there was a significant effect of corn types on plant damage (Table 3, Fig. 5). Non-GM corn exhibited the highest number of overall plant damage, while *Bt* corn had the lowest (Fig. 5).

**Table 2.** Percentage of plants with damage symptoms associated with ACB (for different plant parts) and non-ACB pests in 19 towns of the Isabela province during the wet growing season of 2010. (ACB-associated symptoms include holes in leaves, stems, cobs and the presence of egg masses, in different corn types).

| Corn Types | Number of fields | Number of plants inspected | Percentage of plants with ACB damage: Leave | Stem | Cob | Egg mass | ACB damage | non-ACB damage |
|---|---|---|---|---|---|---|---|---|
| Non-GM corn | 63 | 12600 | 13.16 | 1.44 | 1.79 | 0.62 | 15.70 | 2.39 |
| *Bt* corn | 30 | 6000 | 6.45 | 0.17 | 0.80 | 0.27 | 6.80 | 2.03 |
| *Bt*HT corn | 91 | 18200 | 6.82 | 0.03 | 0.35 | 0.18 | 7.00 | 1.44 |
| HT corn | 14 | 2800 | 9.46 | 0.25 | 1.04 | 0.46 | 11.21 | 1.00 |
| **Total** | **198** | **39600** | **8.97** | **0.52** | **0.92** | **0.35** | **10.03** | **1.80** |

**Table 3.** Results of regression analysis showing the estimate and standard error of ACB-, non-ACB and overall plant damage per field, varying significantly per corn type. (P values: *** = P<0.001, ** = P<0.01, * = P<0.05, (*) = P<0.10).

| | Estimate | Std. Error | t-value | Pr(>\|t\|) | $R^2$ | p-value |
|---|---|---|---|---|---|---|
| **ACB damage** | | | | | **0.043** | **0.009**** |
| *Intercept* | | | | | | |
| - Non-GM corn | 2.886 | 0.139 | 20.732 | <2e-16 *** | | |
| *Contrast with intercept* | | | | | | |
| - *Bt* corn | -0.508 | 0.245 | -2.073 | 0.040* | | |
| - *Bt*HT corn | -0.587 | 0.181 | -3.243 | 0.001** | | |
| - HT corn | -0.101 | 0.326 | -0.310 | 0.757 | | |
| **Non-ACB damage** | | | | | **0.011** | **0.158** |
| *Intercept* | | | | | | |
| - Non-GM corn | 0.617 | 0.128 | 4.822 | $2.87e^{-06}$ *** | | |
| *Contrast with intercept* | | | | | | |
| - *Bt* corn | 0.506 | 0.225 | 2.249 | 0.026* | | |
| - *Bt*HT corn | 0.191 | 0.166 | 1.148 | 0.252 | | |
| - HT corn | 0.063 | 0.300 | 0.208 | 0.835 | | |
| **Overall damage (either by ACB or non-ACB)** | | | | | **0.044** | **0.008**** |
| *Intercept* | | | | | | |
| - Non-GM corn | 3.079 | 0.114 | 26.978 | <2e-16 *** | | |
| *Contrast with intercept* | | | | | | |
| - *Bt* corn | -0.438 | 0.201 | -2.180 | 0.031* | | |
| - *Bt*HT corn | -0.495 | 0.148 | -3.338 | 0.001** | | |
| - HT corn | -0.173 | 0.268 | -0.645 | 0.520 | | |



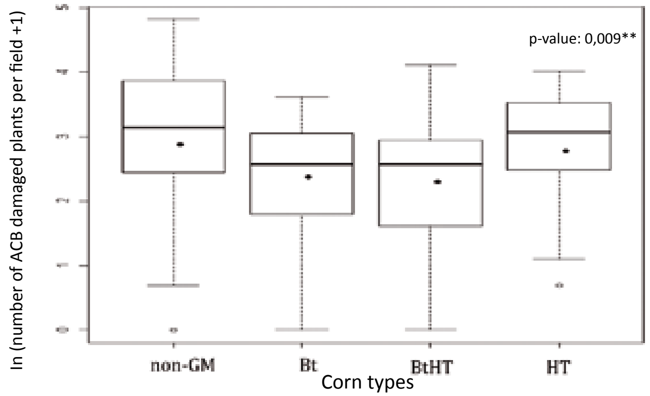


**Figure 3.** Numbers of plants with damage associated with ACB infestation, for the different corn types. The dark line is the median line, the black dot is the mean, the box encloses the interquartile range and the whiskers show the full range, with outliers shown as circles. Values were ln(x+1) transformed. P-values: *** = P<0.001, ** = P<0.01, * = P<0.05, (*) = P<0.10).

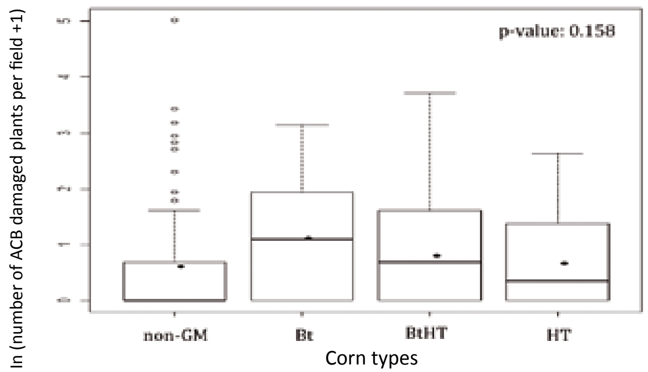


**Figure 4.** Number of plants with damage associated with non-ACB infestation, for the different corn types. The dark line is the median line, the black dot is the mean, the box encloses the interquartile range and the whiskers show the full range, with outliers shown as circles. Values were ln(x+1) transformed. P-values: *** = P<0.001, ** = P<0.01, * = P<0.05, (*) = P<0.10).

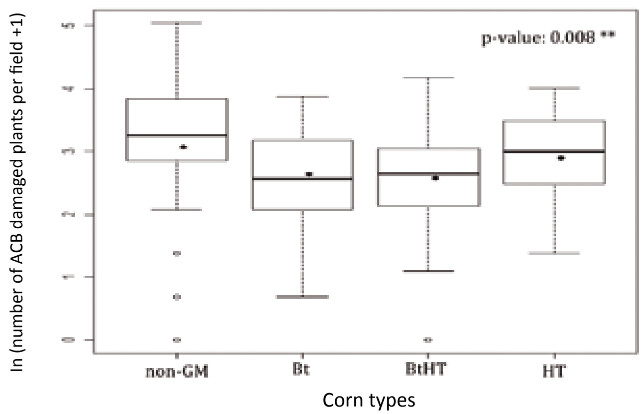


**Figure 5.** Overall plant damage caused by ACB and non-ACB pests for the different corn types. The dark line is the median line, the black dot is the mean, the box encloses the interquartile range and the whiskers show the full range, with outliers shown as circles. Values were ln(x+1) transformed. P-values: *** = P<0.001, ** = P<0.01, * = P<0.05, (*) = P<0.10).

*ACB damage in different plant parts*

Among the four corn types, non-GM corn consistently showed high numbers of leaf, cob and stem holes (Table 4, Fig.6) associated with ACB damage. Apart from leaf damage, the *Bt*HT corn showed the smallest proportion of ACB-damaged plants (Table 4).

**Table 4.** Estimate and standard error of plant part damage by ACB for different corn types, based on regression analyses. (P values: *** = P<0.001, ** = P<0.01, * = P<0.05, (*) = P<0.10).

| | Esti-mate | Std. Error | t-value | Pr(>\|t\|) | $R^2$ | p-value |
|---|---|---|---|---|---|---|
| **Leaf** | | | | | **0.041** | **0.012*** |
| *Intercept* | | | | | | |
| - Non-GM corn | 2.844 | 0.118 | 24.079 | $<2e^{-16}$ *** | | |
| *Contrast with intercept* | | | | | | |
| - *Bt* corn | -0.490 | 0.208 | -2.355 | 0.019* | | |
| - *Bt*HT corn | -0.478 | 0.154 | -3.113 | 0.002** | | |
| - HT corn | -0.150 | 0.277 | -0.543 | 0.588 | | |
| **Cob** | | | | | **0.066** | **0.001**** |
| *Intercept* | | | | | | |
| - Non-GM corn | 0.836 | 0.105 | 7.983 | $1.22e^{-13}$*** | | |
| *Contrast with intercept* | | | | | | |
| - *Bt* corn | -0.427 | 0.184 | -2.316 | 0.022* | | |
| - *Bt*HT corn | -0.552 | 0.136 | -4.049 | $7.44e^{-05}$*** | | |
| - HT corn | -0.276 | 0.246 | -1.121 | 0.264 | | |
| **Stem** | | | | | **0.017** | **0.094(*)** |
| *Intercept* | | | | | | |
| - Non-GM corn | 0.258 | 0.067 | 3.865 | 0.000 *** | | |
| *Contrast with intercept* | | | | | | |
| - *Bt* corn | -0.089 | 0.118 | -0.757 | 0.450 | | |
| - *Bt*HT corn | -0.219 | 0.087 | -2.518 | 0.012* | | |
| - HT corn | -0.110 | 0.157 | -0.700 | 0.484 | | |
| **Egg masses** | | | | | **0.009** | **0.191** |
| *Intercept* | | | | | | |
| - Non-GM corn | 0.376 | 0.074 | 5.047 | $1.03e^{-06}$ *** | | |
| *Contrast with intercept* | | | | | | |
| - *Bt* corn | -0.104 | 0.131 | -0.796 | 0.427 | | |
| - *Bt*HT corn | -0.208 | 0.097 | -2.147 | 0.033* | | |
| - HT corn | -0.055 | 0.175 | -0.313 | 0.755 | | |

The proportional test for *Bt* corn and non-*Bt* corn showed that the number of holes in leaves of infested plants was lower for non-*Bt* corn than for *Bt* corn. The number of ACB-associated holes in cobs and stems was smaller in *Bt* corn. The proportion of plants with egg masses was nearly significantly lower in *Bt* corn (Table 5).

**Table 5.** Proportional test for damage to leaves, stems and cobs as well as the presence of egg masses, for *Bt* and non-*Bt* corn

| Variables | sample estimates | Chi-square | p-value |
|---|---|---|---|
| Leaf | | 184.6035 | < 2.2e-16*** |
| (non-*Bt*) | 0.843 | | |
| (*Bt*) | 0.975 | | |
| Cob | | 106.0798 | < 2.2e-16*** |
| (non-*Bt*) | 0.083 | | |
| (*Bt*) | 0.009 | | |
| Stem | | 24.6602 | 6.838e-07*** |
| (non-*Bt*) | 0.113 | | |
| (*Bt*) | 0.066 | | |
| Egg masses | | 3.1937 | 0.07392(*) |
| (non-*Bt*) | 0.040 | | |
| (*Bt*) | 0.029 | | |

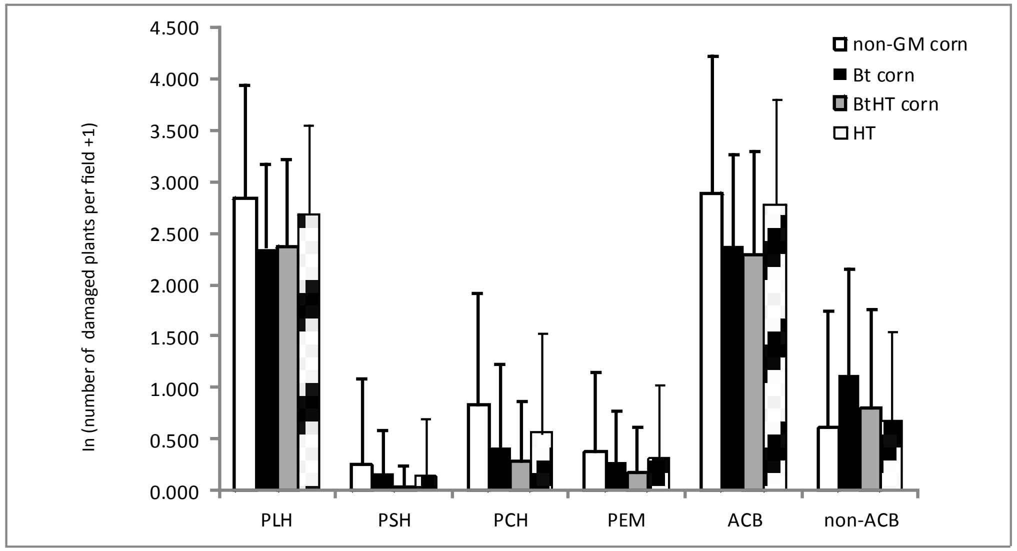


**Figure 6.** Numbers of damaged plants per field due to ACB and non-ACB pests, for different parts of corn plants (i.e. numbers of holes in leaves, stems or cobs, or number of egg masses. P-values: *** = P<0.001, ** = P<0.01, * = P <0.05, (*) = P <0.10.

*Pest damage and spatial variables*

Regression analyses of corn type and spatial variables as explanatory variables showed that the numbers of plants with ACB damage, with non-ACB damage and overall damage were hardly affected by spatial factors, except for the distance to the river. This was true for both ACB and non-ACB damage (Table6), but the effect of distance to river did not appear in the overall damage.

This is because ACB damage was positively correlated with the distance to the river, while non-ACB damage was negatively correlated with this distance (Table 7). Important here, however, is the fact that correction for spatial variables did not cause the significant effect of corn type on ACB and overall damage to disappear, nor did it introduce a significant effect on non-ACB damage.

*ACB vs. non-ACB*

A negative correlation was found between ACB and non-ACB plant damage (Table 7). Table 8 shows that ACB damage was lower in fields with higher non-ACB damage, and vice versa. The interactions between corn types were not significant (Table 8, Figs 7a and b).

**Table 6.** Results of multiple regression analyses showing the sum square values of plant damage per response variable (i.e. ACB, non-ACB or overall damage) per corn type. Spatial attributes of the cornfields, such as distance to the river, elevation and absolute and relative locations, were the explanatory variables included in the statistical model. (P values: *** = P<0.001, ** = P<0.01, * = <0.05, (*) = P<0.10).

| Between corn types | Sum Sq | Mean Sq | F-value | Pr(>F) | $R^2$ | p-value |
|---|---|---|---|---|---|---|
| **ACB damage** | | | | | **0.068** | **0.009**** |
| - Corn types | 14.450 | 4.817 | 4.052 | 0.008 ** | | |
| - Relative location | 0.009 | 0.004 | 0.004 | 0.996 | | |
| - Distance to river | 5.188 | 5.188 | 4.364 | 0.038 * | | |
| - Elevation | 3.030 | 3.030 | 2.549 | | | |
| - Latitude (X) | 0.079 | 0.079 | 0.067 | | | |
| - Longitude (Y) | 1.021 | 1.021 | 0.859 | 0.355 | | |
| - Interaction (X*Y) | 5.169 | 5.169 | 4.348 | 0.038 * | | |
| **Non-ACB damage** | | | | | **0.067** | **0.010*** |
| - Corn types | 5.416 | 1.805 | 1.857 | 0.138 | | |
| - Relative location | 6.804 | 3.402 | 3.499 | 0.032* | | |
| - Distance to river | 4.759 | 4.759 | 4.894 | 0.028* | | |
| - Elevation | 0.230 | 0.230 | 0.237 | | | |
| - Latitude (X) | 0.866 | 0.866 | 0.891 | | | |
| - Longitude (Y) | 2.633 | 2.633 | 2.708 | 0.102 | | |
| - Interaction (X*Y) | 2.665 | 2.665 | 2.741 | 0.099(*) | | |
| **Overall damage (either by ACB or non-ACB)** | | | | | **0.066** | **0.011*** |
| - Corn types | 9.938 | 3.313 | 4.132 | 0.007** | | |
| - Relative location | 0.902 | 0.451 | 0.563 | 0.571 | | |
| - Distance to river | 0.461 | 0.462 | 0.576 | 0.449 | | |
| - Elevation | 2.258 | 2.258 | 2.817 | 0.095(*) | | |
| - Latitude (X) | 0.012 | 0.012 | 0.014 | | | |
| - Longitude (Y) | 3.181 | 3.181 | 3.967 | 0.048* | | |
| - Interaction (X*Y) | 2.394 | 2.394 | 2.986 | 0.086(*) | | |





**Table 7.** Correlation matrix. Pearson coefficients in the upper right part of the matrix, P-values in the lower left part.

| Variables | | Pearson's correlation coefficient | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ACB | Non-ACB | Relative location | Longitude | Latitude | Distance to river | Elevation |
| P-values | ACB | | -0.341 | -0.001 | -0.015 | -0.014 | 0.121 | 0.133 |
| | Non-ACB | 8.973e-07 | | 0.010 | -0.139 | 0.034 | -0.120 | 0.125 |
| | Relative location | 0.984 | 0.892 | | -0.766 | -0.474 | 0.216 | -0.113 |
| | Longitude | 0.836 | 0.051 | 2.2e-16 | | 0.346 | -0.252 | -0.224 |
| | Latitude | 0.849 | 0.636 | 1.646e-12 | 5.963e-07 | | -0.431 | 0.168 |
| | Distance to river | 0.088 | 0.093 | 0.002 | 0.000 | 2.249e-10 | | -0.064 |
| | Elevation | 0.063 | 0.080 | 0.113 | 0.001 | 0.018 | 0.371 | |

**Table 8.** Results of regression analyses showing differences between ACB and non-ACB plant damage. The table represents the output of the minimal models selected after stepwise regression analyses. Values were ln(x+1) transformed. P-values: *** = P<0.001, ** = P<0.01, * = <0.05, (*) = P<0.10.

| Between corn types | Sum Sq | Mean Sq | F-value | Pr(>F) | $R^2$ | p-value |
|---|---|---|---|---|---|---|
| **ACB-damage** | | | | | **0.157** | **1.649e-07***** |
| -Non-ACB | 29.178 | 29.178 | 27.152 | 4.809e-07*** | | |
| -Corn types (Bt and non Bt) | 7.890 | 7.890 | 7.342 | 0.007** | | |
| -Non-ACB* Corn Types | 0.374 | 0.374 | 0.348 | 0.556 | | |
| -Elevation | 6.384 | 6.384 | 5.940 | 0.016 | | |
| **Non-ACB damage** | | | | | **0.138** | **2.738e-06***** |
| -Non-ACB | 23.832 | 23.832 | 26.542 | 6.372e-07 | | |
| -Corn types (Bt and non Bt) | 0.347 | 0.347 | 0.387 | 0.535 | | |
| -Non-ACB* Corn Types | 0.120 | 0.120 | 0.134 | 0.715 | | |
| -Elevation | 4.348 | 4.348 | 4.842 | 0.029* | | |
| -Longitude | 4.151 | 4.151 | 4.623 | 0.033* | | |

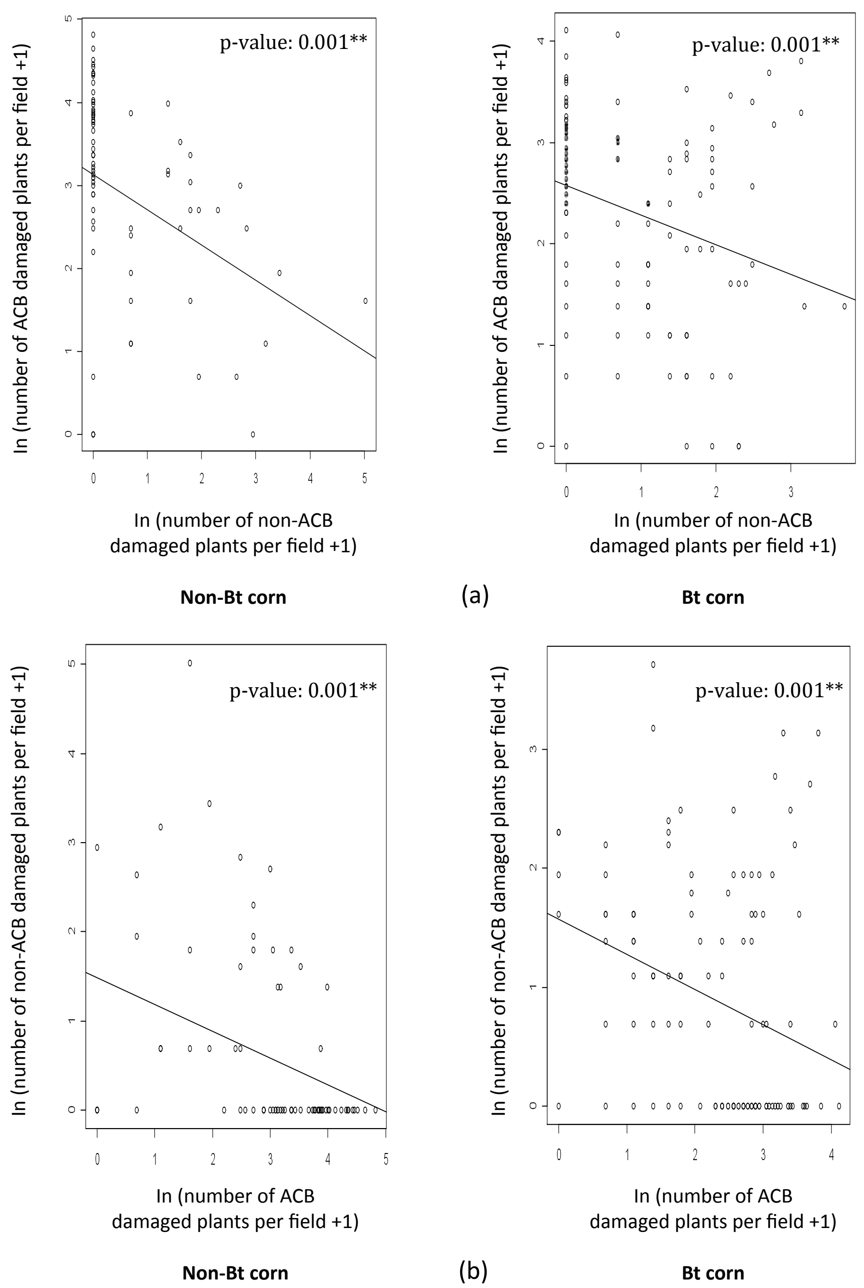


**Figure 7.** Interaction correlation graphs of the number of plants with damage associated with ACB and non-ACB pests. Two models were used to test the negative correlations between ACB and non-ACB: (1) Model A, ACB as response variable and non-ACB as explanatory variable in the case of non-Bt corn (left) and *Bt* corn (right); (2) Model B, non-ACB as response variable and ACB as explanatory variable in the case of non-*Bt* corn (left) and *Bt* corn (right). Values are ln(x+1) transformed numbers of damaged plants per field.





**Discussion**

*Performance of GM corn*

In the present study, the high percentage of ACB-damaged plants in non-GM corn (with 15.7% of all plants showing some kind of damage) indicates that ACB is still a major pest problem in Isabela. Damage was considerably less in GM corn types, with both *Bt* and *Bt*HT corn showing a 44% reduction in ACB damage. ACB damage clearly dominated the overall damage (i.e. the damage attributed to ACB as well as to non-ACB pests). Specifically, the overall plant damage in *Bt* cornfields was significantly less than in non-*Bt* cornfields. This indicates the potentially beneficial effects of adopting *Bt* corn to reduce plant damage caused by corn pests, especially ACB.

The present findings confirm those of studies by He *et al*. (2006) and Thomson *et al*. (2010), which found evidence of reduced ACB damage in *Bt* toxin-containing corn. Field trials done by He *et al*. (2006) proved that Mon *Bt* corn is highly resistant to ACB damage. In the Philippines, field evaluation of two TC1507 *Bt* corn varieties by Thomson *et al*. (2010), assessed percentage reduction in borer tunnels and leaf injury at 99 and 84% respectively. The high discrepancy in the reduction in percentage of ACB damage between the present study and the findings of Thomson *et al*. (2010) could be mainly caused by differences in the cry protein compositions of the *Bt* corn varieties used. In the present study, all *Bt* corn types tested contained Cry1Ab protein, while Thomson *et al*. (2010), used *Bt* corn varieties containing Cry1F protein; hence, different cry proteins may vary in efficacy against ACB.

*Bt plant parts susceptible to ACB*

Comparison between the leaf, stem and cob parts revealed that the leaves are the most susceptible to ACB attack. Voracious leaf feeders such as ACB larvae mostly attack corn leaf because it is easy to chew and softer than stem and cob. Although the Cry1Ab protein was found in higher concentrations in leaves, its concentration fluctuates upon maturity, whereas the stems have a stable concentration of Cry1Ab protein throughout the growth stages (Székács *et al*., 2010b). As the present survey was done between tasselling and maturity growth stages, it is highly possible that the Cry1Ab concentration in the leaves was diminished, making them more susceptible to ACB attack. The lower level of cob damage in infected plants that was noted in *Bt* corn as compared with non-*Bt* corn implies that economic loss in *Bt* corn is minimal even when plants are infested by ACB. Likewise, kernel damage, which may trigger the growth of fungi causing mycotoxin contamination, could also be prevented by using *Bt* corn (Ajangaa and Hillocks, 2000).

Among corn types, *Bt*HT corn was much less affected by ACB damage to leaves, cobs and stems. As regards egg masses, there was no significant effect of corn type. This could indicate that adult ACBs do not recognise *Bt* corn plants. The present study thus provides field evidence that ACB oviposition preference was not affected by corn containing *Bt* toxin.

*ACB resistance to GM corn*

Some studies (Bourguet *et al*., 2003; Farinós *et al*., 2004; Alcantara *et al*., 2011; Tan *et al*, 2011) found little or no borer resistance to *Bt* toxin. In the present study, although *Bt* and *Bt*HT plants had the lowest numbers of damaged plants, they were not free from ACB infestation, as shown in Table 2. A very low (1-2%) incidence of ACB-damaged plants was anticipated, yet ACB proved to inflict damage to 6.8 and 7% of *Bt* and *Bt*HT plants, respectively (Table 2).

The first- and second-instar larval stages of ACB are leaf feeders, and they could die after feeding on corn plants with *Bt* toxin. If they survive the *Bt* toxin, they continue to develop into the third-, fourth- and fifth-instar larval stages. At these stages, the ACB larvae with their well-developed and powerful mandibles are able to inflict great damage to corn plants and produce large holes in stems and leaves. The present survey found mostly large holes in *Bt* corn plants, indicating that ACB larvae emerged, grew and transformed into third-, fourth- and fifth-instar stages. The fact that the ACB spent some of their lifetime eating on *Bt* corn plants means that they survive the effects of *Bt* toxin at least for some time.

Archer *et al*. (2000) evaluated four *Bt* varieties, Mon810 (Cry1Ab), *Bt*11 (Cry1Ab), *Bt* 176 (Cry1Ab) and CBH354 (Cry9c), for the control of south-western and European corn borers. Whereas the second generation of corn borers were controlled well by Mon810, *Bt*11 and CBH354, *Bt* 176 exhibited susceptibility to corn borers, and the damage rate was comparable with that in non-*Bt* hybrids. This shows that corn borers could potentially develop tolerance to some commercially available *Bt* corn hybrids, as in the case of event *Bt* 176.

Borers can develop a certain degree of resistance when continuously exposed to *Bt* toxin. The research by Huang *et al*. (2007) provided the first evidence that a target pest may develop resistance to *Bt* toxin. The study by van Rensburg (2007) found that substantial numbers of African stem borer larvae survived over the entire trial period, although the mean larval mass was less on Mon810 *Bt* corn than on non-*Bt* corn. Recent findings by Kojima *et al*. (2010) suggest that ACB could easily adapt to the chemical defences of its host plants. In view of these findings, plus the continued large-scale monocropping of *Bt* corn in the Philippines, there is a great risk of ACB resistance to *Bt* toxin developing via physiological adaptation. In particular, the presence and persistence of ACB in *Bt* cornfields is an indication that ACB could develop some adaptive characteristics, such as the development of resistance genes to Cry1Ab similar to those of the sugarcane borer, *Diatraea saccharalis* (Huang *et al.,* 2007).

*Non-ACB pests in the GM corn environment*

Pests such as the corn leafhopper *Cicadulina bimaculata* (Evans), corn earworm *Helicoverpa armigera* (H?bner) and corn leaf aphid *Rhopalosiphum maidis* Fitch (Litsinger *et al*., 2007) can reduce yields, although they are considered to be of less importance than ACB damage. In the present study, similar species of non-ACB pests may have caused higher plant damage in *Bt* corn. This suggests that *Bt* and *Bt*HT are only effective against ACB and do not offer defence against non-ACB pests. To some extent, this contradicts the findings of Pilcher *et al*. (1997) and Lynch *et al*. (1999), who found that *Bt* corn could partially control and reduce damage by pests such corn earworm and armyworm (*Pseudaletia unipunctata*).



Likewise, the high negative correlation between ACB and non-ACB damage might indicate that ACB is negatively influenced by the occurrence of non-ACB pests, or the other way around. In addition, the significant number of non-ACB-damaged plants in *Bt* fields suggests that non-ACB pests are resistant to *Bt* toxin. Yet this also indicates that, when ACB is continuously absent from *Bt*-protected fields, niche intrusion could take place and secondary pests could emerge and come to dominate in time. Further, the low percentage of ACB-damaged plants in *Bt* cornfields where significantly higher numbers of non-ACB pests were observed could also mean that ACB can detect the presence of competitors in the plants and look for other habitats with fewer or no competitors (Bernasconi *et al.,* 1998).



The trend lines in Figures 7a and b indicate that corn type was not responsible for the negative correlations between ACB and non-ACB pests, and that other factors should be considered. This is supported by Table 8, which shows no interaction effects between pest damage and corn type. There is no reason to assume that the high abundance of non-ACB pests is due to corn type; hence, at this stage the existence of secondary pest development cannot be confirmed.

**Conclusion**

In conclusion, *Bt* and *Bt*HT corn hybrids containing the Cry1Ab protein performed well in Isabela Province. This was manifested by the significant reduction (by 44%) in ACB damage in inspected *Bt* cornfields. The fact that ACB inflicted greater damage in *Bt* leaves than in stems and cobs indicates that leaves are more susceptible to ACB attack. The lower level of cob damage by ACB in *Bt* fields could mean smaller economic losses even when such fields are infested by ACB. The occurrence of ACB in *Bt* and *Bt*HT cornfields, although at a moderate and insignificant level, could, however, indicate the potential development of resistance to *Bt* toxin.

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