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

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Field assessment of the impact of genetically modified (GM) corn cultivation and its associated agricultural practices on in-field invertebrate populations in the Philippines

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To be submitted

Abstract

Simplified agricultural practices, involving no tillage, no insecticide inputs and lower human labor requirements, are now the preferred corn farming system and have been generally adopted in the Philippines. This system involves cultivation of genetically modified (GM) corn such as insect-resistant *Bacillus thuringiensis* (*Bt*) corn and *Bt* plus herbicide-tolerant (*Bt*HT) corn. Adopting GM corn cultivation removes the need for insecticides and enables labor-intensive manual weeding to be replaced by methods involving herbicides. This is assumed to yield superior economic returns. Yet, the effect of GM corn on biodiversity is an as yet unresolved issue, especially in a biodiversity hotspot like the Philippines. The GM effects on biodiversity were studied in a six-hectare field experiment in Cabagan, Isabela, The Philippines, during the 2009 dry and wet cropping seasons, in order to evaluate the short-term effect of GM corn (i.e. *Bt* and *Bt*HT) on the community of in-field invertebrates. Our findings showed that the total invertebrate abundance, surface dweller abundance and species richness of surface dwellers and soil dwellers were significantly higher in non-GM cornfields than in *Bt* and *Bt*HT cornfields. Insecticide-sprayed non-GM cornfields harbored more invertebrates than unsprayed *Bt* or *Bt*HT cornfields. Chemical weeding may adversely affect invertebrates in both glyphosate- and Gramoxone-sprayed fields. Higher number of invertebrates was found in fully weeded fields (100% weed cover). Finally, this study provides evidence that complex agricultural farming in non-GM cornfields is more favorable for in-field invertebrates than simplified farming systems involving GM corn.

Introduction

Biodiversity decline in agroecosystems is often linked to modern agricultural practices (Flohre *et al*., 2011). One of the key aspects of such practices is the use of chemicals to increase yield and avert crop losses due to pests and diseases. However, pesticides have deleterious effects on humans and biodiversity (for reviews see Stoate *et al*., 2001; Geiger *et al*., 2010; Waggoner *et al*., 2011; Yadav and Sehrawat, 2011).

Advocates of agricultural biotechnology claim that genetically modified (GM) corn can potentially mitigate the impact of agricultural intensification, and that *Bacillus thuringiensis* (*Bt*) corn offers the best alternative to traditional insecticide treatment for the control of major agricultural pests (Chen *et al.,* 2008). The expectations of high yields, lower pesticide inputs and lower time investments attributed to GM corn have caused an upsurge in its adoption among major corn-producing countries. Among 55 countries having adopted *Bt* corn, 25, including the Philippines, publicly promote the commercial adoption of GM corn. In 2009, transgenic crops covered 135 million hectares in 25 countries (James, 2009). In the US alone, the area covered by *Bt* corn reached more than 22.2 million hectares, making up 63% of US crops (Hutchison *et al*., 2010). In the Philippines, the three GM corn varieties readily available on the market are *Bt* insect–resistant corn, Round-up Ready (RR)/herbicide-tolerant (HT) corn and stacked genes *Bt*HT corn. In 2009, GM corn acreage grew to 350,000 ha (James, 2009). In addition, the area planted with *Bt*HT corn in 2008 was 200,000 ha, which is a 300% increment from the 63,000 ha in 2007 (Poquiz, 2009). Currently, more transgenic herbicide-tolerant varieties are about to be introduced, which may imply a rapid advancement of the "*Gene Revolution*" in the Philippines.

The effectiveness of *Bt* endotoxin as a biopesticide has made *Bt* corn a popular variety for corn-growing areas with widespread infestations of the Asian corn borer (ACB), *Ostrinia furnacalis* Guenée. *Bt* corn's efficiency in killing corn borers is facilitated by the constitutive expression of the Cry1Ab endotoxin in all parts of the *Bt* plant (Wilkinson *et al.,* 1997; Roh *et al.,* 2007; Burkness *et al*., 2001). Although *Bt* toxin is harmful to ACB, it is still considered an environment-friendly toxin because it is highly specific, with few known adverse effects on non-target species (Glare and O'Callaghan, 2000). The *Bt* toxin has proved to be non-toxic to several non-target arthropods and pests in various laboratories (Sims and Martin, 1997; Escher *et al.,* 2000; Saxena and Stotzky, 2001; Alfageme *et al*., 2010; Bakonyi *et al*., 2011;) and field studies (Bhatti *et al*., 2005a; Bhatti *et al*., 2005b; Rauschen *et al*., 2009).

Herbicide-tolerant corn is protected from glyphosate by its genetically built-in ESP (5-enolpyruvylshikimate-3-phosphate synthase) cDNA, which was isolated from a glyphosate-tolerant petunia cell culture line (Padget *et al.,* 1995). This makes the HT plants tolerant to four times the concentration of glyphosate required to kill weeds. Glyphosate, a broad-spectrum herbicide is degradable (Cerdeira and Duke, 2006) and presents a limited risk of surface- and ground-water pollution, due to sorption onto charged soil minerals (Borggaard and Gimsing, 2008). Furthermore, HT corn was reported to benefit farmland biodiversity (Firbank & Forcella, 2000; Dewar *et al*., 2003; Freckleton *et al.*, 2004). Delayed spraying in HT corn enables weeds to grow, creating a microhabitat and food resource for arthropods and associated species. Finally, HT corn promotes no-tillage agriculture, which reduces soil erosion and the risk of surface water pollution, and produces more diverse soil biota (Holland, 2004), thus providing substantial environmental benefits (Cerdeira and Duke, 2006). However, some biodiversity conservationists do not support the idea that GM corn is the ideal option, as many vital issues still need to be addressed and many remain unresolved.

As regards *Bt* corn, there is the potential occurrence of *Bt* gene introgression (Arias and Rieseberg, 1994; Mikkelson *et al.,* 1996; Yin and Stotzky, 1997). The reinforced *Bt* gene function could transform other organisms into harmful, invasive and hard to eliminate species (Shen, 2006). The probable expression of new proteins apart from the intended transgenic products might produce unpredictable mechanisms such as pleiotropic effects (Uberlacker *et al.*, 1996). In addition, there is the potential development of resistance to *Bt* toxin (Altieri, 2000). A meta-analysis by Marvier *et al.* (2007) found that non-target organisms are more abundant in *Bt* corn yet, when compared to non-*Bt* corn with no insecticide application, some non-target groups are less abundant. Finally, other issues that still require answers are the non-target effects of *Bt* toxin and loss of biodiversity due to monocropping of GM crops (Linder and Schmitt, 1995; Arriola and Ellstrand, 1997; Altieri, 2000; Dutton *et al*., 2003; Andow and Hilbeck, 2004).

As for HT corn, the US National Research Council (NRC) recently reported the development of so-called "glyphosate-resistant weeds" near GM cornfields (Benaning, 2010), which may lead to the development of super-weeds such as *Amaranthus palmeri* (Brown *et al.,* 1996; Altieri, 2000; Hammond, 2010). There is a high risk that weed population composition may shift to naturally resistant species. A good example is the development of resistance to glyphosate by the population of horseweed, *Conyza canadensis* L (Owen and Zelaya, 2005). Pimentel *et al*. (1989) reported glyphosate to be toxic to some non-target beneficial organisms such as spiders, mites, carabids, coccinellid beetles and earthworms, as well as to aquatic organisms, including fish. In addition, it may accumulate in fruits and tubers due to its slow metabolic degradation in plants (Altieri, 2000).

The claim by proponents that GM crops will result in minimal use of pesticide remains questionable. U.S. government data from 1994 to 2005 reveal a 15-fold increase in the use of glyphosate since the nationwide adoption of HT crops (Anonymous, 2009).

We studied these opposing claims by conducting field experiments in the Philippines in order to reveal the effects of GM corn on biodiversity in actual farm scenarios. These effects can be assessed by comparing the abundance and species richness of invertebrates in farm-managed *Bt*, *Bt*HT and non-GM cornfields. The importance of in-field invertebrates for agroecosystems (Firbank *et al*. 2003), and their potential direct exposure to toxin and/or pest control chemicals (herbicides/insecticides), make them an interesting fauna category to focus on. The adoption of transgenic corn will lead to changes in agricultural practices, which may become very different from or similar to management practices using traditional corn hybrid varieties. One potential effect is that agricultural practices such as insecticide application, weed control (i.e. timing/frequency of application, types of herbicide and methods of weeding) and human/animal labor may be reduced due to widespread use of GM corn.

Our study intended to provide an overview of the effects of the adoption of GM corns and associated practices on in-field invertebrate populations. Hence, the experiment had two objectives: (1) exploring the potential impacts of *Bt* and *Bt*HT corn on invertebrate populations as compared to iso-hybrid non-GM corn; and (2) investigating the impact on invertebrate populations of alterations to the crop management systems associated with the adoption of *Bt* and *Bt*HT corn.

Methods

Study area

The study involved a field-based experiment conducted at five different sites in the northeast Isabela Province of the large island of Luzon, The Philippines, located in the town of Cabagan (17°25.650N; 121° 45.883E). Experimental fields covering 6 hectares were planted in the villages of Catabayungan (twice), Ugad, Garita, Cansan and Cubag for two consecutive cropping seasons in 2009. To ensure that there would be no residual effects of transgenic crops, we selected fields that had never been cultivated with any type of transgenic corn. In the case of the Catabayungan site, one corn type was planted on the same plot for the whole duration of the study.

Experimental design

The experimental design used a split-plot randomized complete block design (RCBD) including two experimental factors, i.e. corn type and agricultural practice. Each of three corn types was used on one-third of each one-hectare experimental cornfield. Different types of agricultural practice (i.e. herbicide and insecticide management and weeding methods) were assigned randomly to subplots within each corn type. Each treatment was replicated four times (Figure 1).

The three corn types that were planted included two transgenic corn hybrids, viz. *Bt* (DeKalb YG) and *Bt*HT (DeKalb YGHT), and a non-genetically modified (non-GM) iso-hybrid corn (DeKalb). A 2 m gap was left in between corn types to prevent edge effects. Hence, each plot was subdivided into ten sub-plots of almost equal size. Each sub-plot corresponded to one treatment, which consisted of a specific combination of agricultural practices and corn type (Figure 1). Within each sub-plot, samples of invertebrates were taken using four pitfalls, four sticky traps and four soil cores per sampling round.

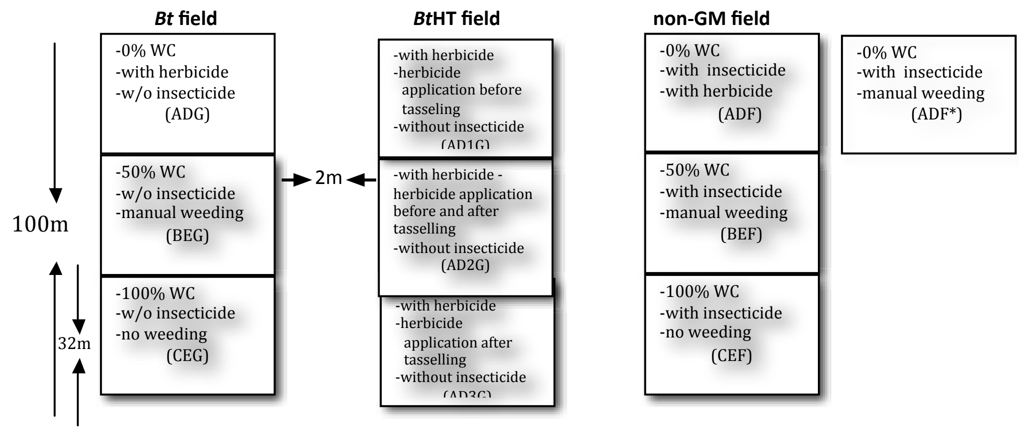

Figure 1. Experimental layout in a one-hectare experimental field. Split plots were used per corn variety. Letter codes were assigned to the plots, referring to treatment combinations of commonly used agricultural practices, per corn variety: AD1G (*Bt*HT corn, with herbicide, no insecticide and post-emergence herbicide application at an early stage), AD2G (*Bt*HT corn, with herbicide, no insecticide, two post-emergence herbicide applications), AD3G (*Bt*HT corn, with herbicide, no insecticide, one post-emergence application after tasselling corn stage), ADG (*Bt* corn, with herbicide, no insecticide, 0% weed cover [WC]), BEG (*Bt* corn, no herbicide, no insecticide, 50% WC), CEG (*Bt* corn, no herbicide, no insecticide, 100% WC), ADF (non-*Bt* corn with herbicide, with insecticide, 0% WC), ADF* (non-GM corn, no herbicide, with insecticide, 0% WC), BEF (non-GM corn, no herbicide, with insecticide, 50% WC), and CEF (non-GM corn, no herbicide, with insecticide, 100% WC).

Sampling techniques

Invertebrate traps and soil sampling points (Fig. 2) were set out along 100-m transect lines, which were laid out in the middle of the fields to prevent potential edge effects (Dively and Rose, 2003). A total of 1,584 pitfall collections, 792 sticky traps, and 264 soil core samples were collected over the entire duration of this study.

Aerial fauna

Our study used a technique similar to that used by Bhatti *et al.* (2005) for trapping foliage arthropods. Yellow sticky traps (8 x 13 cm) were used to collect aerial dwelling invertebrates. From January to March 2008, 120 traps (12 per field) were set up in Pilig Abajo for each corn growth stage. Then, from March to April 2008, a total of 120 traps were set up at all three sites (40 traps per site at three sites per field, 10 m apart). Sticky cards were attached to the tips of bamboo sticks (length: 91 cm) and placed vertically, 5-10 cm above the surface. The traps remained in the field for two consecutive nights and were collected on the third day.

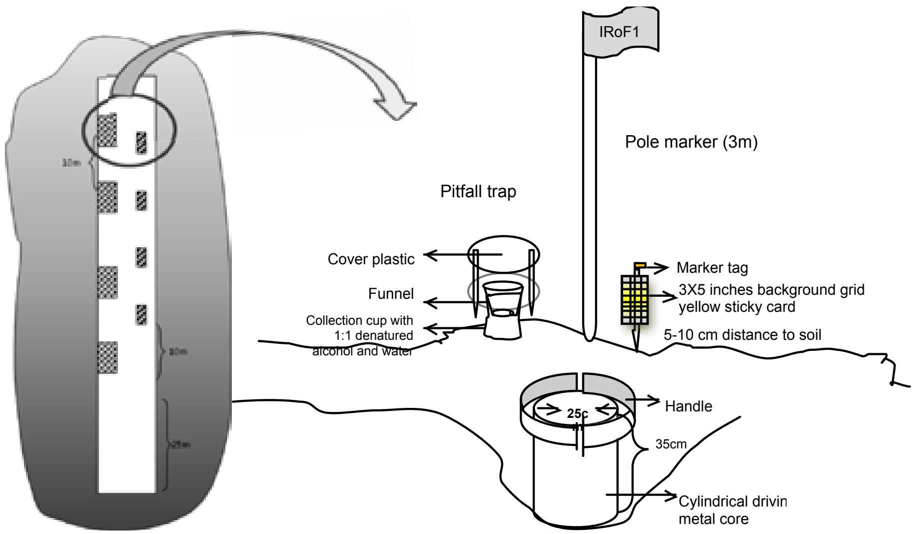

Figure 2. Locations of pitfall, sticky traps and soil core sample collection points in a transect line established in the middle of each farm.

Surface fauna

A pitfall sampling technique was used to sample surface-dwelling invertebrates (Ivanov and Keiper, 2009). The pitfall traps were constructed using 1.5 L plastic bottles. Each trap had a uniform mouth (diameter: 9.5 cm, length: 15 cm) and was buried with the mouth at ground surface level. From January to March 2008, 40 traps (four per field) were set up in Pilig Abajo for each corn growth stage. In March 2008, a total of 120 traps were set up at all three sites (40 traps per site at three sites per field, 10 m apart). A 1:1 mixture of water and alcohol was used as temporary preserving medium in the field. Traps were monitored thrice a week to ensure enough preserving medium remained in the traps. Pitfall traps were collected at the end of each week for two consecutive weeks.

Soil fauna

Soil fauna was sampled using soil cores (Ahmad *et al*., 2005). In each field, four soil cores (10 m apart) were collected in March 2008. Samples were collected close to the corn plant roots (2 cm away from the plant base), using a cylindrical metal core sampler (diameter: 25 cm; height: 35 cm). The samples were taken to the Botanical Laboratory of Isabela State University in Cabagan, Isabela, for sorting and safekeeping. Soil fauna were extracted by gradually pulverizing the soil using bare hands. The specimens were collected then stored in containers with denatured alcohol for preservation and, at a later stage, identification.

Taxonomic identification of invertebrate species

Initial identification and documentation of the specimens collected was performed at the Fauna Laboratory of Isabela State University, Cabagan, Isabela. Final species validation was done by experts of the Entomology division of the National Museum in Manila, Philippines. Each individual invertebrate was identified down to species level, whenever possible. Identified species were categorized into the following functional guilds: herbivores, detritivores, omnivores, predators, and parasitoids.

Weed cover

The effects of weed cover (WC) on invertebrates were determined by manipulating the weed cover in the field, either through manual weeding or by using herbicides. The variants used were zero (0), fifty (50) and one hundred (100) percent WC. The herbicides used to maintain the zero weed cover subplots were glyphosate for *Bt*HT and Gramoxone (paraquat) for *Bt* and non-GM corns. Controlled manual weeding was used to maintain the 50% WC subplots, while for 100% WC subplots, weeds were allowed to grow unrestricted. (Figure 3)

Pesticide management

Fields were treated with the traditional mode of liquid spraying, using an aluminum knapsack sprayer only for all non-*Bt* corn plots. Furadan (carbofuran) insecticide was applied twice (i.e. before and after the tasselling stage of the corn) to protect plants during the periods in which they are vulnerable to ACB infestation. But both *Bt* and *Bt*HT plots were left unsprayed, on the assumption that both corn types have the genetically built-in *Bt* Cry1Ab endotoxin, which is effective against ACB. All non-transgenic plots were insecticide-sprayed, while all *Bt* and *Bt*HT corn plots were insecticide-free.

Two post-emergence herbicide applications were applied before the plants reached a height of 0.10 to 0.20 m (Everman, 2010). Likewise, two herbicide Gramoxone (paraquat) applications were used for *Bt* and non-*Bt* plots. Additional herbicide was applied especially during the wet season, when weeds grow fast. Three different application timings were used for the *Bt*HT cornfields, to assess the potential effects of herbicide application on invertebrates. Treatments used were (1) one post-emergence treatment at the vegetative stage; (2) two post-emergence treatments during early and late development stages; and (3) one post-emergence treatment in the late growth stage.

Figure 3. Experimental plots showing varying percentages of weed cover (% WC): (a) 0% WC, herbicide-sprayed (upper left); (b) 0% WC, manually weeded (upper right); (c) 50% WC, manually weeded (lower left) and; (d) 100% WC or zero tillage (lower right).

Response variables

The response variables used to gauge the potential short-term, farm-scale effect of transgenic corn and its associated agricultural practices were the mean per-trap values of the abundance and species richness of all invertebrates and the abundance of individual guilds. Each invertebrate was counted, and whenever possible, identified to species level. All identified species were categorized into five groups based on their ecological role (guild), viz. herbivores, omnivores, parasitoids, predators and detritivores.

Statistical analysis

We performed data analyses using R Stat version 2.7. All tables present Wald tests for the explanatory covariates (fixed factors), based on the parameter estimates and their standard errors (Quinn and Keough, 2007). Mixed regression analysis i.e. Generalized Linear Mixed Model (GLMM) and Restricted Maximum Likelihood (REML) were used. GLMM was used to deal with the random and fixed factors. The random predictors in the model are plots, sites, cropping seasons and sampling method. The fixed factors were corn types and agricultural practices such as weed management, insecticide management and weeding methods. REML was employed to deal with the multi-level samplings with plots nested within corn stages and sites, which in turn were nested within cropping seasons. Either lognormal or Poisson models were applied, whichever fitted best, as shown by the histogram residuals. All data presented are natural log-transformed values of the mean per trap. A significance level of 0.05 was used (Rao and Balakrishnan, 1999). Only results with significant values are presented and discussed, unless otherwise specified. Lastly, we performed posthoc analysis using Bonferroni correction; the significance test is shown in the tables. This analysis was done by dividing the significance level by the number of models within that specific analysis.

Results

GM

Abundance and species richness

The pooled data from two consecutive cropping seasons related to 83,684 individuals, mainly comprising 55,226 aerial, 27,374 surface-dwelling and 1,084 soil-dwelling invertebrates (Table 1). Of all these invertebrates, 29% (23,875), 30% (24,782) and 42% (35,027) were collected in *Bt*, *Bt*HT and non-GM cornfields, respectively. The abundance of all invertebrates captured in non-GM corn plots was 19% (11,152) higher than in *Bt* corn and 17% (10,245) higher than in *Bt*HT cornfields. The mean (±SE) abundance per trap revealed the same pattern and showed significant differences between corn types (Table 2).

The total numbers of species captured using sticky cards, pitfall traps and soil cores were 105, 82 and 27, respectively (Table 1). The highest mean number of species captured per trap was observed in non-GM cornfields, followed by *Bt* cornfields and *Bt*HT cornfields (Table 2).

Table 1. Total counts of invertebrate assemblage collected in GM and non-GM cornfields in Isabela Province, Philippines during the dry and wet growing seasons of 2009. FG= Functional guild (H=herbivore, O=omnivore, Pa=parasitoid, Pr=predator, De=detritivores); ID= Types of invertebrate dwellers (AD= aerial dweller, SF = soil fauna, SD = surface dweller).

Species	ID	FG	Abundance
Empoasca fabae	AD	H	21348
Draeculacephala mollipes	AD	H	6015
Phaenicia sericata	AD	De	5010
Drosophila melenogaster	AD	De	4300
Micraspis discolor	AD	Pr	3802
Sciara sp.	AD	De	3367
Phormia regina	AD	De	1246
Aulacophora sp.	AD	H	1221
Erythroneura vitis	AD	H	1136
Rhysella nitida	AD	Pa	895
Cheilomenes sexmaculatus	AD	Pr	893
Olibrus sp.	AD	De	762
Oncocephalus confusus	AD	Pr	665
Liodontemerus sp.	AD	Pa	555
Germalus elegantulus	AD	Pr	450
Blatella germanica	AD	O	442
Phytodictus vulgaris	AD	Pa	349
Fannia sp.	AD	De	226
Amerimicromus sp.	AD	Pr	219
Tettigella viridis	AD	H	217
Aphidalestes sp.	AD	H	204
Salticus sp.	AD	Pr	179
Trichiohelcon sp.	AD	Pa	175
Onukia onuki	AD	H	148
Scirtes sp.	AD	H	147
Tetragnatha mandibulata	AD	Pr	127
Heppelates sp.	AD	H	103
Dermestres sp.	AD	H	97
Euphorocera claripennis	AD	Pa	94
Oxyopes sp.	AD	Pr	70
Teleogryllus sp.	AD	O	70
Ostrinia furnacalis	AD	H	46
Chelonus texanus	AD	Pa	42
Pteromalus sp.	AD	Pa	41
Euxesta sp.	AD	De	40
Myrmacea maxilosa	AD	Pr	38
Adelphocoris ropidus	AD	H	37
Solenopsis pergundei	AD	O	32
Ragoletis pomonella	AD	H	31
Syrphus ribesii	AD	O	30
Pyraus sp.	AD	H	28
Clerada sp.	AD	Pr	26
Pholcus phalingoides	AD	Pr	26
Diatraes sp.	AD	Pa	24
Digitress sp.	AD	Pa	24
Apanteles thomsoni	AD	Pa	19
Scolypopa australis	AD	H	16
Bruchus sp.	AD	H	14
Anolepsis longipes	AD	O	13
Clavicornaltica sp.	AD	H	10
Cercion calamorum	AD	Pr	9
Cexius angustatus	AD	H	9

Species	ID	FG	Abundance
Ploiaria regina	AD	Pr	9
Anacharis sp.	AD	Pa	8
Brachymeria sp.	AD	Pa	8
Scaphoideus festivus	AD	H	7
Andrena sp.	AD	Pr	6
Chortoicetus sp.	AD	H	6
Cofana spectra	AD	H	6
Apion sp.	AD	H	5
Hylemya platura	AD	H	5
Metoponium sp.	AD	H	5
Allenobius fasciatus	AD	O	4
Diacampus sp.	AD	Pa	4
Fornax sp.	AD	H	3
Goelerucella maculicollis	AD	H	3
Isidromus sp.	AD	Pa	3
Labidurata truncatu	AD	H	3
Nomadacris gutturosa	AD	H	3
Onthropagus sp.	AD	H	3
Opion sp.	AD	H	3
Adelocera sp.	AD	H	2
Ceriagrim liefiricki	AD	Pr	2
Cymidis sp.	AD	Pr	2
Iphiulax sp.	AD	Pa	2
Monomorium minimum	AD	O	2
Oceantatus quadrimaculatus	AD	H	2
Oecleus borealis	AD	H	2
Phocambe disparis	AD	Pa	2
Platyzosteria nitidella	AD	O	2
Solenopsis globularia	AD	O	2
Trachelus tabidus	AD	H	2
Agyra leucocephala	AD	Pr	1
Aufidus sp.	AD	H	1
Bubekia fallax	AD	Pa	1
Cocinella sp.	AD	Pr	1
Crematogaster clara	AD	O	1
Cyclas formicarius	AD	H	1
Dolichopus sp.	AD	Pr	1
Endomychus sp.	AD	Pr	1
Felisacus glabratus	AD	Pr	1
Gastrolinoides sp.	AD	H	1
Gracilaria sp.	AD	H	1
Hesperus sp.	AD	Pr	1
Heterothrips sp.	AD	H	1
Hister sp.	AD	Pr	1
Leptocorisa acuta	AD	Pr	1
Lucilia ilustris	AD	De	1
Neozelobia sp.	AD	De	1
Omyrus sp.	AD	Pa	1
Orosicus argentatus	AD	H	1
Pnyxia sp.	AD	De	1
Prosevania punctata	AD	Pa	1
Trigonotoma sp.	AD	H	1
Ugyops sp.	AD	H	1
Total individual of aerial dwellers =			**55,226**
Total species of aerial dwellers =			**105**

Species	ID	FG	Abundance
Oxyopes sp.	SD	Pr	4547
Solenopsis globularia	SD	O	4475
Albonemobius fasciatus	SD	O	3796
Monomorium minimum	SD	O	3084
platozosteria nitida	SD	O	2646
Drosophila melenogaster	SD	De	1748
Megacephala sp.	SD	O	1180
Myara sp.	SD	O	744
Hesperus sp.	SD	Pr	721
Megasellia sp.	SD	Pr	667
Pselaphus sp.	SD	Pr	491
Anolepsis longipes	SD	O	429
Adelocera sp.	SD	H	392
Labidura truncatu	SD	H	262
Sciara sp.	SD	De	257
Salticus sp.	SD	Pr	191
Cylisticus convexus	SD	De	188
Parcoblatta spp.	SD	O	185
Trigonoma sp.	SD	H	168
Draeculacephala mollipes	SD	H	163
Euxoa excellens	SD	H	140
Sippuna sp.	SD	Pr	112
Fannia sp.	SD	De	105
Adrisa sp.	SD	Pr	82
Myrmarachne maxillosa	SD	Pr	52
Solenopsis invicta	SD	O	51
Heppelates sp.	SD	H	49
Cymindis sp.	SD	Pr	38
Teleogylus sp.	SD	O	37
Brachymeria sp.	SD	Pa	24
Geophilus sp.	SD	Pr	24
Musca domestica	SD	De	20
Felisacus glabaratus	SD	Pr	16
Onthophagus sp.	SD	H	16
Lumbricoides sp.	SD	De	15
Empoasca fabae	SD	H	14
Aulacophora sp.	SD	H	13
Phitemera bicincta	SD	De	13
Americamus sp.	SD	Pr	12
Cocinella sp.	SD	Pr	12
Ostrinia furnacalis	SD	H	12
Rhysellia nitida	SD	Pa	12
Thiara sp.	SD	De	12
Cylas formicarius elegantulus	SD	H	10
Pachyndola sp.	SD	O	10
Tetigella viridis	SD	H	10
Oxidus gracilis	SD	De	9
Geophilomorpha sp.	SD	Pr	8
Pardosa pseudoammulata	SD	Pr	8
Callibaetis sp.	SD	O	7
Orosorius argentatus	SD	Pr	7
Rhagoletes cingulata	SD	H	7
Cleoporus variabilis	SD	H	6
Dolichopus sp.	SD	Pr	6
Glyptotermes sp.	SD	De	6
Coproporus sp.	SD	Pr	5
Deleaster yokoyamai	SD	Pr	5
Chelonus sp.	SD	Pa	4
Liodomentus sp.	SD	Pa	4
stegonium panicerum	SD	Pr	4

Species	ID	FG	Abundance
Syrphus ribessi	SD	O	4
Tineola bissellula	SD	H	4
Crematogaster minutissima	SD	O	3
Micraspis discolor	SD	Pr	3
Orchesia sp.	SD	H	3
Tetragnatha mandibulata	SD	Pr	3
Blatella sp.	SD	O	2
Cheilomenes sexmaculatus	SD	Pr	2
Cletus trigonus	SD	H	2
Coniotis sp.	SD	H	2
Diamma bicolour	SD	Pa	2
Geophilus erectus	SD	Pr	2
Phobocampe disparis	SD	Pa	2
Dictyotus caenosus	SD	Pr	1
Hister sp.	SD	Pr	1
Labidura riparia	SD	H	1
Metoponium sp.	SD	H	1
Nomadacris gutturosa	SD	H	1
Pinophilus sp.	SD	Pr	1
Scirtes sp.	SD	H	1
Talia sp.	SD	O	1
Trogoderma sp.	SD	H	1
Total individual of surface dwellers =			**27,374**
Total species of surface dwellers =			**82**

Species	ID	FG	Abundance
Lumbricoides sp.	SF	De	248
Pheiodole megacephala	SF	O	238
Phitemera bicincta	SF	De	165
Solenopsis invicta	SF	O	98
Solenopsis globularia	SF	O	55
Thiara sp.	SF	De	52
White grub	SF	H	46
Adrisa sp.	SF	Pr	27
Platozosteria nitida	SF	O	25
Dictyotus caenosus	SF	H	21
Oxyopes sp.	SF	Pr	20
Cylisticus convexus	SF	De	17
Geophilomorpha sp	SF	Pr	10
Geophilus sp.	SF	Pr	9
Titanolabis colossea	SF	De	8
Oxidus gracilis	SF	De	7
Sericesthis geminata	SF	Pr	7
Pselaphus sp.	SF	Pr	6
Salicus sp.	SF	Pr	6
Adelocera sp.	SF	H	4
Labiduratu truncatu	SF	H	4
Hirudiea medicinalis	SF	De	3
Pinophilus sp.	SF	Pr	3
Onthophagus sp.	SF	H	2
Cymindis sp.	SF	Pr	1
Phaeri sericata	SF	De	1
Syrphus ribesii	SF	O	1
Total individual of soil fauna (SF) =			**1,084**
Total species of soil fauna =			**27**

*GRAND TOTAL OF INVERBRATE INDIVIDUALS ACCOUNTED = **83,684***

Table 2. Results of the mixed regression analyses (REML) of the abundance and species richness of invertebrates per dwelling category, with corn variety as fixed factor and plot within site and cropping season as random factors. Mean per trap was ln (x+1) transformed. Sign B: significance after Bonferroni correction: *** = equivalent to p<0.001; * = equivalent to p<0.05.

	Bt	*Bt*HT	non-GM	Model	Chi-sqr	p	Sign B
	Mean ± SE	Mean ± se	Mean ± se				
Abundance	3.057±0.0456	3.056±0.046	3.162±0.0386	Lognormal	10.230	0.006	*
Species Richness	1.905±0.024	1.896±0.024	1.941±0.0196	Poisson	3.296	0.192	
Abundance per dwelling category							
Aerial	4.156 ±0.042	4.137 ±0.054	4.076 ±0.042	Lognormal	3.553	0.169	
Surface	2.762 ±0.039	2.783 ±0.038	2.979 ±0.036	Lognormal	16.598	0.000	***
Soil	1.235 ±0.107	1.180 ±0.105	1.335 ±0.092	Lognormal	1.419	0.492	
Species Richness per dwelling category							
Aerial	2.465 ±0.016	2.437 ±0.023	2.441 ±0.017	Poisson	0.267	0.875	
Surface	1.796 ±0.018	1.795 ±0.018	1.848 ±0.016	Poisson	6.343	0.042	
Soil	0.769 ±0.058	0.781 ±0.058	0.904±0.054	Poisson	5.255	0.072	

Abundance and species richness per dwelling category

Of the three types of invertebrates in terms of dwelling (aerial, surface-dwelling and soil-dwelling), only the abundance and species richness of surface-dwelling species showed differences between corn types (Table 1). Among surface dwellers, predators seemed to be more abundant in non-GM corn plots, whilst aerial predators were more abundant in *Bt*HT corn plots (Table 3).

Table 3. Results of the mixed regression analyses (REML) of the abundance of herbivore, detritivore, omnivore, predator and parasite invertebrates in general and for the three dwelling categories, with corn variety as fixed factor and plot within site and cropping season as random factors. Mean per trap was ln(x+1) transformed. Sign B: significance after Bonferroni correction: ** = equivalent to p<0.01; * = equivalent to p<0.05.

	Bt Mean se	*Bt*HT Mean se	non-GM Mean se	Model	Chi-sqr	P	Sign B
GUILDS							
- Omnivore	1.353±0.047	1.334±0.046	1.420±0.044	Lognormal	3.432	0.180	
- Herbivore	1.474±0.061	1.443±0.064	1.484±0.052	Lognormal	1.586	0.452	
- Predator	1.425±0.038	1.489±0.039	1.469±0.035	Lognormal	2.268	0.322	
- Parasitoid	0.392±0.027	0.388±0.026	0.408±0.024	Lognormal	0.930	0.628	
- Detritivores	1.392±0.050	1.330±0.049	1.405±0.042	Lognormal	4.644	0.098	
Aerial Dwellers							
- Omnivore	0.395±.0384	0.323±0.031	0.382±0.035	Lognormal	2.352	0.309	
- Herbivore	3.474±0.052	3.517±0.062	3.423±0.050	Lognormal	2.859	0.240	
- Predator	1.869±0.067	1.953±0.068	1.651±0.061	Lognormal	20.454	3.633^{e-05}	**
- Parasitoid	1.144±0.051	1.117±0.050	1.176±0.043	Lognormal	0.884	0.643	
- Detritivores	2.780±0.054	2.638±0.064	2.702±0.051	Lognormal	5.284	0.071	
Surface Dwellers							
- Omnivore	2.113±0.048	2.124±0.048	2.262±0.047	Lognormal	5.186	0.075	
- Herbivore	0.543±0.032	0.454±0.030	0.569±0.027	Lognormal	7.838	0.020	
- Predator	1.401±0.043	1.470±0.043	1.594±0.043	Lognormal	12.073	0.002	*
- Parasitoid	0.019±0.006	0.029±0.008	0.029±0.007	Lognormal	1.234	0.540	
- Detritivores	0.696±0.042	0.682±0.040	0.751±0.036	Lognormal	2.581	0.275	
Soil Dwellers							
- Omnivore	0.427±0.094	0.420±0.093	0.322±0.079	Lognormal	1.057	0.589	
- Herbivore	0.132 ±0.036	0.161±0.041	0.240±0.046	Lognormal	4.338	0.114	
- Predator	0.208±0.039	0.190±0.040	0.292±0.051	Lognormal	4.017	0.135	
- Parasitoid	-	-	-		-	-	
- Detritivores	0.714±0.010	0.644±0.095	0.780±0.083	Lognormal	1.480	0.477	

GM and its associated agricultural practices

Insect management

Our results show that the mean abundance of invertebrates per trap was higher in insecticide-sprayed fields than in unsprayed fields, and this pattern was found for all guilds. Insecticide-sprayed cornfields featured the highest mean number of species per trap (Table 4).

Table 4. Results of the mixed regression analyses (REML) of the abundance and species richness of all invertebrates and of the individual guilds, with insect management type as fixed factor and plot within site and cropping season as random factors. Mean per trap was ln(x+1) transformed. Sign B: significance after Bonferroni correction: ** = equivalent to p<0.01.

	Insect Management (non-GM, *Bt* and *Bt*HT)					
	no insecticide (*BT*&*Bt*HT)	with insecticide (non-GM)	Model	Chi-sqr	P	Sign B
	Mean se	Mean se				
Abundance	3.056±0.032	3.162 ±0.039	Lognormal	10.224	0.001	**
Species Richness	1.900±0.017	1.941 ±0.020	Poisson	3.187	0.074	
- Omnivore	1.344±0.032	1.420 ±0.043	Lognormal	3.282	0.070	
- Herbivore	1.458±0.044	1.484±0.052	Lognormal	0.803	0.370	
- Predator	1.457±0.027	1.469±0.035	Lognormal	0.121	0.728	
- Parasitoid	0.390±0.019	0.408±0.024	Lognormal	0.906	0.341	
- Detritivores	1.361±0.035	1.405±0.042	Lognormal	2.034	0.154	

Weed management

Herbicide-sprayed and unsprayed plots

An analysis of the invertebrate abundance on herbicide-sprayed and unsprayed cornfields showed that this abundance was higher in unsprayed than in herbicide-sprayed cornfields (Table 5). The omnivore guild was found to be more abundant in unsprayed cornfields (Table 5).

Table 5. Results of the mixed regression analyses of the abundance and species richness of all invertebrates and of the individual guilds, with weed management type as fixed factor and plot within site and cropping season as random factors. Mean per trap was ln(x+1) transformed. Sign B: significance after Bonferroni correction: ** = equivalent to p<0.01.

	Weed Management (non-GM, *Bt* and *Bt*HT)		Model	Chi-sqr	P	Sign B
	no herbicide	with herbicide				
	Mean se	Mean se				
Abundance	3.146±0.034	3.051±0.036	Lognormal	8.984	0.003	**
Species Richness	1.930±0.018	1.903±0.019	Poisson	1.829	0.176	
- Omnivore	1.426±0.038	1.322±0.036	Lognormal	7.027	0.008	**
- Herbivore	1.472±0.047	1.465±0.049	Lognormal	0.072	0.789	
- Predator	1.458±0.031	1.465±0.030	Lognormal	0.047	0.828	
- Parasitoid	0.399±0.021	0.396±0.021	Lognormal	0.034	0.854	
- Detritivores	1.403±0.038	1.354±0.038	Lognormal	2.595	0.107	

Gramoxone vs. glyphosate

The abundance of invertebrates in glyphosate-sprayed cornfields was equal to that in Gramoxone-sprayed fields, as was their species richness (Table 6). Of the different guilds, predators differed between the herbicide types in that they were more abundant in glyphosate-sprayed cornfields (Table 6).

Table 6. Results of the mixed regression analyses of the abundance and species richness of all invertebrates and of the individual guilds, with the type of herbicide as fixed factor and plot within site and cropping season as random factors. Mean per trap was ln(x+1) transformed.

	Types of herbicide (non-GM, *Bt* and *Bt*HT)		Model	Chi-sqr	p
	Glyphosate (*Bt*HT)	Gramoxone (non-GM &*Bt*)			
	Mean se	Mean se			
Abundance	3.056±0.047	3.048±0.047	Lognormal	0.113	0.736
Species Richness	1.896±0.024	1.898±0.024	Poisson	0.351	0.553
- Omnivore	1.334±0.046	1.278±0.050	Lognormal	0.857	0.354
- Herbivore	1.443±0.064	1.491±0.062	Lognormal	0.620	0.431
- Predator	1.488±0.038	1.395±0.040	Lognormal	3.868	0.049
- Parasitoid	0.388±0.027	0.398±0.028	Lognormal	1.924^{e-06}	0.999
- Detritivores	1.330±0.049	1.381±0.049	Lognormal	1.048	0.306

Timing and frequency of application

Timing and frequency of herbicide application produced no effects in terms of the abundance and species richness of all invertebrates and the individual guilds (Table 7).

Table 7. Results of the mixed regression analyses of the abundance and species richness of all invertebrates and of the individual guilds, with timing and number of post-emergence herbicide applications as fixed factor and plot within site and cropping season as random factors. Mean per trap was ln(x+1) transformed.

	Frequency and period of herbicide application (*Bt*HT)			Model	Chi-sqr	P
	once, early	once, late	twice			
	Mean se	Mean se	Mean se			
Abundance	3.137±0.080	3.026±0.080	3.005±0.082	Lognormal	4.632	0.099
Species Richness	1.919±0.040	1.925±0.041	1.845±0.043	Poisson	4.298	0.117
- Omnivore	1.425±0.083	1.294±0.077	1.284±0.080	Lognormal	3.572	0.168
- Herbivore	1.475±0.113	1.443±0.108	1.410±0.110	Lognormal	1.066	0.587
- Predator	1.507±0.067	1.495±0.066	1.465±0.069	Lognormal	0.387	0.824
- Parasitoid	0.400±0.047	0.407±0.048	0.358±0.043	Lognormal	1.570	0.456
- Detritivores	1.316±0.084	1.392±0.086	1.280±0.083	Lognormal	2.912	0.233

Weeding methods

The abundance of invertebrates differed for the different weeding methods. Chemical weeding appeared to be unfavorable for invertebrates, while manual weeding led to a higher mean abundance, but the highest value was obtained by no weeding (Table 8). Of the different guilds, omnivores and detritivores were found to be more abundant in manual weeding and no-weeding plots, respectively (Table 8).

Table 8. Results of the mixed regression analyses of the abundance and species richness of all invertebrates and of the individual guilds, with weeding method as fixed factor and plot within site and cropping season as random factors. Mean per trap was ln(x+1) transformed. Sign B: significance after Bonferroni correction: * = equivalent to p<0.05.

	Methods of Weeding (non-GM, *Bt* and *Bt*HT)			Model	Chi-sqr	P	Sign B
	chemical weeding	manual weeding	no weeding				
	Mean se	Mean se	Mean se				
Abundance	3.051±0.036	3.144±0.045	3.148±0.054	Lognormal	8.991	0.011	*
Species Richness	1.903±0.018	1.925±0.023	1.938±0.028	Poisson	2.132	0.344	
- Omnivore	1.322±0.036	1.432±0.051	1.418±0.057	Lognormal	7.095	0.029	
- Herbivore	1.465±0.049	1.476±0.060	1.466±0.074	Lognormal	0.136	0.934	
- Predator	1.465±0.030	1.427±0.039	1.503±0.051	Lognormal	2.455	0.293	
- Parasitoid	0.396±0.021	0.400±0.027	0.398±0.034	Lognormal	0.036	0.982	
- Detritivores	1.354±0.038	1.370±0.049	1.451±0.060	Lognormal	6.095	0.047	

The 50% weed cover (WC) found to exhibit the highest numbers of invertebrates. Of the different guilds, omnivore and detritivore abundance exhibited slight differences depending on the percentage of WC. Omnivores and detritivores were abundant in 50% WC and 100% WC plots, respectively (Table 9).

Table 9. Results of the mixed regression analyses of the abundance and species richness of all invertebrates and of the individual guilds, with percentage of weed cover (%WC) as fixed factor and plot within site and cropping season as random factors. Mean per trap was ln(x+1) transformed.

	Percent Weed Cover (%WC) (*Bt* and non-GM)			Model	Chi-sqr	p
	Zero WC	50% WC	100% WC			
	Mean se	Mean se	Mean se			
Abundance	3.135±0.045	3.232±0.054	3.202±0.053	Lognormal	3.745	0.053
Species Richness	1.954±0.022	1.976±0.027	1.975±0.027	Poisson	1.383	0.240
- Omnivore	1.353±0.049	1.487±0.065	1.427±0.057	Lognormal	2.742	0.098
- Herbivore	1.543±0.063	1.558±0.076	1.526±0.077	Lognormal	0.010	0.921
- Predator	1.466±0.038	1.467±0.048	1.526±0.052	Lognormal	1.576	0.209
- Parasitoid	0.424±0.028	0.420±0.034	0.418±0.035	Lognormal	0.009	0.924
- Detritivores	1.408±0.051	1.424±0.062	1.476±0.062	Lognormal	2.751	0.097

Discussion

Invertebrates and GM (Bt and BtHT) corn

The results of our previous study (Afidchao *et al.*, Chapter 4) of more than two years of transgenic cornfield cultivation indicated that transgenic *Bt* and *Bt*HT corns could adversely affect the abundance and species richness of invertebrates. One of the plausible reasons for this phenomenon is the accumulation of *Bt* toxin in the corn environment over time. The present study, covering two cropping seasons, yielded consistent results supporting the findings of the previous study. Similar findings were obtained for both *Bt* and *Bt*HT.

The findings indicate that the *Bt* protein not only affects the target pest but also other non-target organisms, which is consistent with findings on seven species of Macrolepidoptera in farmland areas in Germany (Schmitz *et al.,* 2003). Likewise, the mini-review of 20 peer-reviewed publications by Lang and Otto (2010) recorded that 52% of the laboratory-based publications and 21% of the field-based observations reported the *Bt* protein having an adverse effect on Lepidopteran caterpillars.

The current study found evidence that short-term cultivation of GM corn can have an impact on the abundance of invertebrates, and specifically found that in a tropical humid environment like the Philippines, non-target organisms were more abundant in insecticide-sprayed non-GM cornfields than in insecticide-free *Bt* cornfields (Table 4). This clearly contradicts the meta-analysis by Marvier *et al.* (2007), which concluded that non-target organisms were more abundant in unsprayed *Bt* cornfields than in sprayed non-*Bt* cornfields. The discrepancy may be due to different ecological conditions in the study areas, as all studies collated in the meta-analysis by Marvier *et al.* (2007) were done in temperate regions. It is highly likely that the behavior, sensitivity and tolerance to toxin differ between non-target species in different agro-ecological conditions. Some related examples are provided by the studies by Garcia (2011) and Römbke *et al*. (2007) on earthworms under tropical and temperate conditions.

Although the effect size seems small, the effects of GM corn on non-target invertebrates imply that GM corn is not environmentally risk-free, and that continued cultivation of such novel varieties could entail a loss of biodiversity. Non-GM cornfields appeared to provide more favorable habitats for in-field invertebrates, emphasizing the need for more sustainable stewardship practices such as the maintenance of non-*Bt* corn refugia (Hutchison, *et al.,* 2010).

Our short-term experiment with the cultivation of GM corns showed that the abundance and species richness of surface dwellers were significantly lower in *Bt* and *Bt*HT GM cornfields than in non-GM fields. This result, combined with the significantly high level of Cry1Ab protein detected in various species of ground beetles by means of ELISA tests (Zwalen and Andow, 2005) suggests that predators are exposed to high *Bt* risks.

Surface-dwelling herbivores seemed also adversely affected in *Bt*HT cornfields, and were more abundant in non-GM cornfields. Herbivores survive in a habitat that consists largely of weeds. Although most pests belong to this guild, their important ecological role, especially in the food web, should not be ignored. Teodorescu and Cogalniceanu (2005) reported that the species richness and number of individuals of aboveground arthropods could be an indicator of biological diversity, and the best indicator of human-induced impacts. Hence, the significant reduction of surface dwellers in our GM fields, consisting mainly of aboveground arthropods, may suggest a disturbed agro-biodiversity that can be linked to the Cry1Ab toxins in the GM fields.

Finally, our previous study (Afidchao *et al*., Chapter 4) shows that long-term cultivation of GM corn does not affect surface dwellers, but that it is the aerial and soil-dwelling invertebrates that are the most affected groups. This appears to contradict our findings in the current study, where surface dwellers were the most adversely affected. This could be due to the fact that drastic changes in the environment may initially impact on surface dwellers, which later become tolerant to toxin and are able to recover their populations, whilst bio-magnification effects of toxin in the corn agro-ecosystem due to continuous GM corn cultivation could not be tolerated by the aerial and soil-dwelling species, thus could affect their population in the long run.

Invertebrates and GM-associated agricultural practices

Insect management

Pesticides are known to have an adverse impact on an ecologically stable agro-biodiversity, by causing massive mortality among non-target taxa and reducing species richness (Geiger *et al*., 2010; Teodorescu and Cogalniceanu, 2005). The most severely affected are the in-field organisms that are directly exposed to the toxin. Reduction of these toxic chemicals could be achieved by zero insecticide application, which is the practice supposed to be adopted by *Bt* and *Bt*HT corn farmers. It is on this premise that many *Bt* toxin containing corn varieties have been promoted as an environmentally friendly alternative to conventional varieties. In the context of the adoption of *Bt* corn, insecticide inputs were expected to be minimized or totally avoided. However, our earlier study (Afidchao *et al.,* Chapter 4) found that *Bt* fields are not free of pesticides and have a lower abundance of invertebrates. In the current study, we found significantly higher abundance and species richness of invertebrates in insecticide-sprayed non-GM cornfields than in unsprayed GM (*Bt* and *Bt*HT) cornfields. This finding explicitly shows that in the short term, insecticide-free *Bt* cornfields are not necessarily more favorable for invertebrates than insecticide-sprayed non-GM cornfields.

Weed management

The weeding method based on herbicide seems to have an adverse effect on the abundance of invertebrates. The most severely affected guilds in *Bt* and *Bt*HT herbicide-sprayed fields were the omnivores and detritivores. This suggests that the use of herbicides such as glyphosate and Gramoxone may in the end have a major ecological impact (Blackburn and Boutin, 2003). A beneficial effect of no weeding was observed specifically among omnivores and detritivores. Weeding per se could have a direct influence in terms of biodiversity loss and reduction of food availability for wildlife within fields (Beringer, 2000)

Glyphosate has been reported to be environment-friendly or risk-free compared to other broad-spectrum herbicides (Knezevic and Cassman, 2003), but our findings do not support this notion. Although the predator guild was slightly more abundant in glyphosate-sprayed fields than in non-sprayed fields, this difference was no longer significant after Bonferroni correction (Table 6). Hence, our study does not support the view that glyphosate-resistant corn may be acceptable as a risk-free alternative.

The absence of weed cover may adversely affect the abundance and species richness of in-field invertebrates, since most of this fauna depends for survival on weeds (serving as food source as well as habitat). This was supported by our findings, as a 50% WC seemed to provide the most suitable habitat for invertebrates, especially those of the omnivore guild, while 100% WC was most suitable for the detritivore guild. This finding is consistent with those of Blumberg and Crossley (1983), who found that no tillage yielded a greater diversity of soil surface arthropods than conventional tillage. In our current study, a major reduction (90%) in weed cover due to the application of herbicides like glyphosate in *Bt*HT corn fields had direct negative effects on in-field invertebrates. In contrast, reduced-tillage (50% WC) or no-tillage (100% WC) agriculture may provide substantial environmental benefits (Cerdeira and Duke, 2006) especially to various invertebrates.

Conclusion

The current study clearly highlights the advantage of non-GM cornfields in terms of the abundance and species richness of all invertebrates and of the ecological guilds. In terms of the agricultural practices assessed, insecticide-sprayed non-GM fields were more favorable for invertebrates than unsprayed GM fields. Our field evaluations, comparing two GM corn varieties, showed that GM *Bt* corn poses less of an environmental risk to invertebrate ecosystem populations than *Bt*HT corn. This was shown by the greater abundance and species richness of all invertebrates and of the different guilds, with the exception of predator species, which were more abundant in the *Bt*HT cornfields. Regimes with no herbicide application generally favor invertebrates, whereas chemical weeding greatly reduces their populations. Finally, our findings provide evidence that neither intensive farming nor farming systems using biotechnology crops safeguard biodiversity, especially that of invertebrates, which play key roles in crop production and balancing the agroecosystem. Although the adoption of *Bt* and *Bt*HT corns may allow simplified production systems, the current study suggests that the more complex production systems associated with the conventional corn varieties are more ecologically sustainable.

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