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A MULTILAYER, LARGE SCALE COMPARISON OF ARTHROPOD COMMUNITIES IN COMMERCIALLY MANAGED Bt AND NON-Bt CORN FIELDS[1]

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ABSTRACT

A 4-year on-farm study in 2006-2009 was conducted to monitor arthropod herbivore abundance in commercial corn fields in Camarines Sur, Philippines. Visual inspection of corn plants was carried out on six (6) separate dates during each cropping season. Principal response curve analysis of herbivore abundance data collected in every planting season showed similar species compositions in Bt- and non-Bt corn. Dominance distribution was represented by nine taxa including corn leaf hopper *Stenocranus pacificus* Kirkaldy, corn leaf aphid *Rhopalosiphum maidis* (Fitch), leaf bug *Riptortus* sp., Acrididae, Agromyzidae, Cicadellidae, pink stem borer *Sesamia inferens* (Walker), Gryllidae and Derbidae. Natural enemies highly associated with Bt corn were *Micraspis discolor* (Fab.), *Proreus* sp., *Solenopsis geminata* (Jerdon) and *Orius tantillus* Motschulsky. The development of pest succession was not detected by correspondence analysis. Taken together, the study showed that long term commercial planting of Bt corn does not have adverse effect on arthropod herbivore communities.

Key words: Bt corn, PRC analysis, dominance structure, pest succession

INTRODUCTION

Bacillus thuringiensis (Bt) is a common soil bacterium that produces crystalliferous proteins with insecticidal properties. Through genetic engineering, several crops expressing Cry proteins have acquired protection from insect damage. One such crop is Bt corn. The Philippines has now become one of the world's major users of the Bt corn technology since its approval by the government for commercial propagation in 2002. The land area planted with Bt corn has reached 500,000 hectares as of the end of 2010 (James 2010). The adoption of Bt corn in the country has significantly increased corn farmers' productivity (Yorobe and Quicoy 2006; Brookes and Barfoot 2008) because of effective control of the Asian corn borer *Ostrinia furnacalis* (Guenee) resulting in superior grain quality.

Secondary ecological effects refer to any impact on non-target or beneficial insects, to food webs and soil microflora and fauna. The impact could be acute or chronic toxicity to predators resulting from ingestion of transgenic Cry protein from herbivorous prey feeding on Bt corn. Beneficial non-target arthropods have an important role in regulating the population of insect pests in corn fields. These arthropods are exposed to transgenic Cry1Ab protein in Bt corn either through bitrophic or tritrophic interaction (Naranjo 2009). A serious consequence of adverse secondary ecological effects might be the elimination of an entire food chain when herbivorous prey is susceptible to insecticidal activity of transgenic Cry protein. It is generally considered, however, that the risk of exposure to transgenic Cry proteins is very low. The present study would like to address the long term impact of exposure of arthropods inside the transgenic corn ecosystem to transgenic Cry1Ab protein expressed in Bt corn.

The objectives of the present study are 1) to compare herbivore communities within Bt and non-Bt corn farms, 2) to determine associations between natural enemies and crop type (i.e., Bt and non-Bt), 3) to compare herbivore dominance distribution within Bt and non-Bt corn farms and 4) to determine occurrence of pest succession in Bt corn farms.

MATERIALS AND METHODS

Experimental Design. A paired-comparison experiment in randomized complete block design was carried out using three pairs of commercial farms located in the towns of Ocampo, Sañgay and Tigaon in the province of Camarines Sur, Philippines (Figure 1). Each pair of farms served as a replicate. The area of each farm is about 1 hectare and the distance between the paired farms is not more than 1 km. Crop types (i.e., Bt corn Dekalb 818 YieldGard and near isoline non-Bt corn Dekalb 818) served as the treatments. Farmers' practice includes the application of the insecticide Larvin® at planting for whorl maggot (*Atherigona oryzae* Malloch) control in all corn planting sites. Experiments were conducted for five consecutive seasons from June 2006 to April 2009.

Sampling. Arthropod counts were determined by visual sampling as described by Dively (2005) with modifications. One hundred corn plants in a "W" pattern that covered the whole farm were sampled in every treatment replicate. All above-ground parts of each plant were carefully examined for arthropods. Both sides of the leaves were examined. Arthropod specimens observed from visual samplings were recorded and identified up to the species level whenever possible. All sampling activities were conducted early in the morning (0600H). Sampling schedule was as follows: 25, 35, 45, 55, 60 and 110 days after planting (DAP), which corresponded with the early vegetative (V4), middle vegetative (V8), late vegetative (V12), reproductive tasseling (RT), reproductive silking (RS) and maturity (M) stage, respectively.

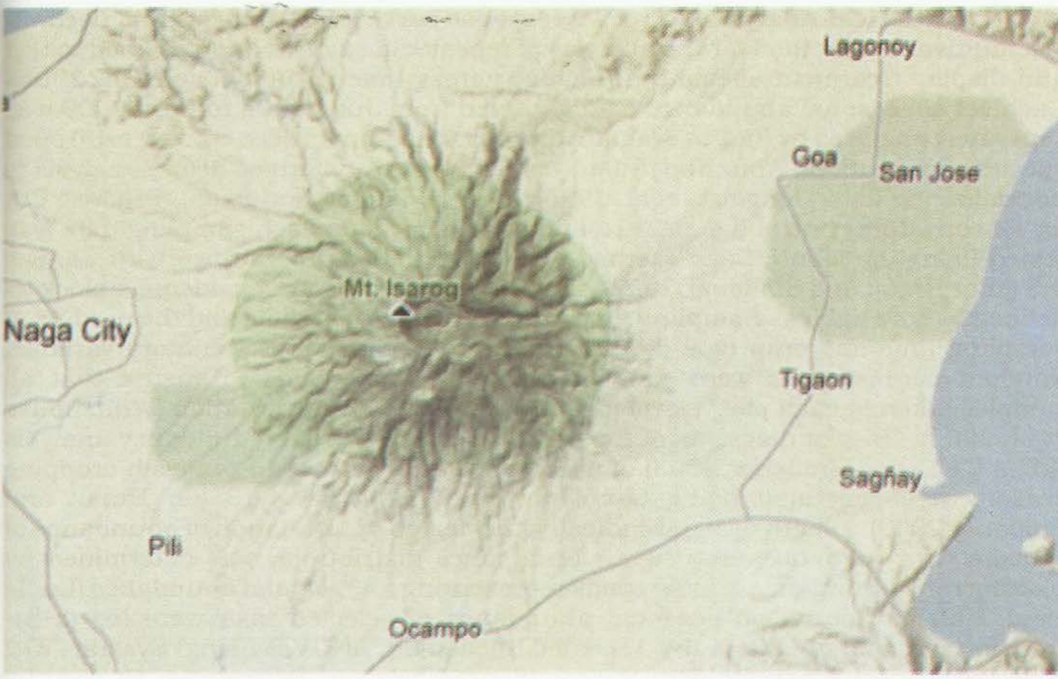


Figure 1. Location map of experimental sites in the towns of Ocampo, Sañgay and Tigaon, Camarines Sur. A pair of commercial farms, consisting of one farm planted with Bt corn and another farm planted with near isoline non-Bt corn was selected in each town. The size of one farm is about one hectare. The distance between farms in each pair was no more than one kilometer. Field experiments were conducted for five consecutive seasons from June 2006 to April 2009.

Table 1. Characteristics of Principal Response Curves of arthropod herbivore communities in selected Bt corn fields in Camarines Sur Province[a]

	Jun-Sep 2006	Jan-Apr 2007	Jun-Sep 2007	Jun-Sep 2008	Jan-Apr 2009
F- value	2.004	1.255	2.967	2.142	1.334
P-value (calculated using Monte Carlo Simulation, 999 permutations)	0.519	0.992	0.319	1.000	0.989
Variance explained by crop type	11.6	8.70	7.30	9.8	7.40
Proportion of this variance explained by Axis 1	46.2	45.0	55.7	53.1	49.1
Variance explained by sampling date	35.6	27.4	65.6	41.4	49.1

[a] Each data in the Table was generated by Principal Response Curve analysis of ln (X+1)-transformed arthropod counts from three pairs (each pair consists of Bt- and non-Bt corn farms) of commercial farms selected for the study in the towns of Ocampo, Sañgay and Tigaon from June 2006-April 2009. Arthropod counts were gathered by visual sampling of 100 corn plants in each farm of every replicate at 25, 35, 45, 55, 60 and 110 days after planting.

Statistical Analysis. Principal response curve (PRC) analysis was used. It is a multivariate method for the analysis of repeated measures and designed to test and display treatment effects that change across time (Whitehouse et al. 2005). Each set of seasonal abundance data gathered from June 2006 to April 2009 was separately analyzed by PRC to evaluate the long term impact of Bt corn on arthropod species composition. Abundance data were ln (x+1)-transformed before analysis to normalize the data (Jongman et al. 1995). The deviation of principal response Cdt for Bt corn from control (i.e. near isoline non-Bt corn) on each sampling date was tested using the Monte-Carlo method (999 permutations) performed with Canoco 4.5 (after Braak and Smilauer 2002). Each pair of farms was considered a block as defined by covariables. Sampling time was treated as a covariate and the product of sampling time and crop type (i.e. Bt- and non-Bt corn) as explanatory variable. Random permutations were conducted for the whole plots to ensure that all samples taken at each plot "travel together" during each permutation (Whitehouse et al. 2005). Bi-plot diagrams of the ordination results from redundancy analysis (RDA) (Lepš and Smilauer 2003) of abundance data gathered from each cropping season were generated using CanoDraw for Windows V.4.13 (ter Braak and Smilauer 2002). Length of arrow indicates the degree of correlation of abundance of a taxon with particular crop type. Dominance distribution was determined by plotting rank abundance of arthropods representing > 1% of total abundance (Leslie et al 2007). Counts on seasonal abundance of selected taxa were log (x+1)-transformed and analyzed by repeated measures ANOVA using Sysstat V12 software. Species assemblages were compared between Bt and non-Bt corn by means of correspondence analysis (CA) as described by Arpaia et al (2007).

RESULTS

Crop type influence on herbivore community composition. Arthropod species compositions within Bt and non-Bt corn farms are similar as revealed by PRC analyses shown in Figure 2. Taxa with weights between -0.5 and +0.5 were not presented because they are likely to show a weak response or a response that is unrelated to the PRC curve (Van den Brink and Ter Braak 1999). The positive species scores indicated how much that species is represented by the PRC and a negative score reflected how much the response looks like the opposite of the PRC. Crop type accounted for 11.6, 8.7, 7.3, 9.8 and 7.4% of the variance for June-September 2006, January-April 2007, June-September 2007, June-September 2008 and January-April 2009, respectively, of which 46.2, 45.0, 55.7, 53.1 and 49.1 %, respectively, of this variance was captured by the first PRC axis (Table 1). A large proportion of variance in the communities was explained by changes during the season because sampling dates accounted for 35.6, 27.4, 65.6, 41.4 and 49.1 % of the variance in the communities for June-September 2006, January-April 2007, June-September 2007, June-September 2008, and January-April 2009 cropping season, respectively.

Herbivore Dominance Distribution. A total of nine herbivore taxa was observed in corn. Derbids, though not the most abundant, were the most common herbivore group observed in all cropping seasons (Figure 3). Dominance

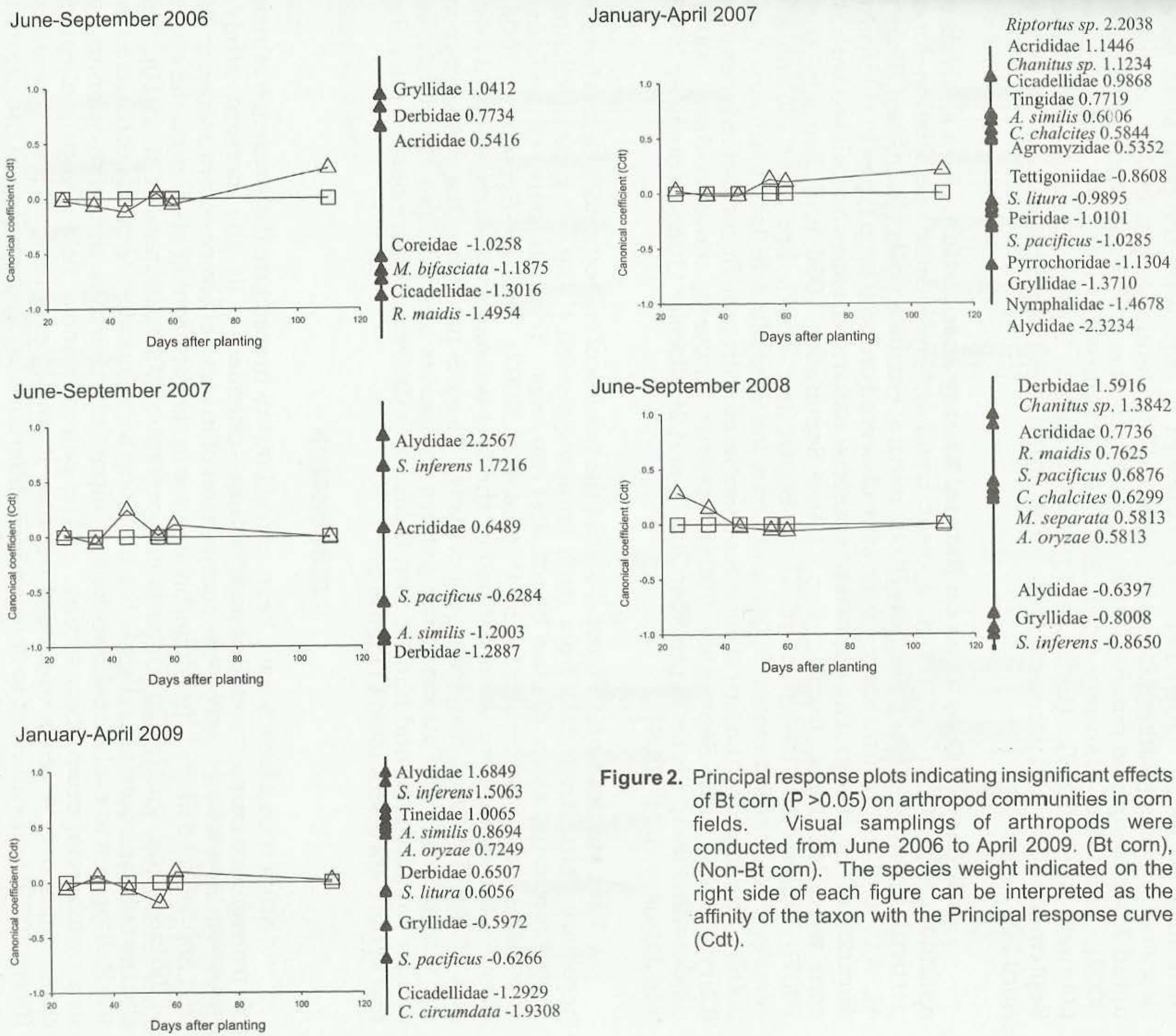


Figure 2. Principal response plots indicating insignificant effects of Bt corn (P >0.05) on arthropod communities in corn fields. Visual samplings of arthropods were conducted from June 2006 to April 2009. (Bt corn), (Non-Bt corn). The species weight indicated on the right side of each figure can be interpreted as the affinity of the taxon with the Principal response curve (Cdt).

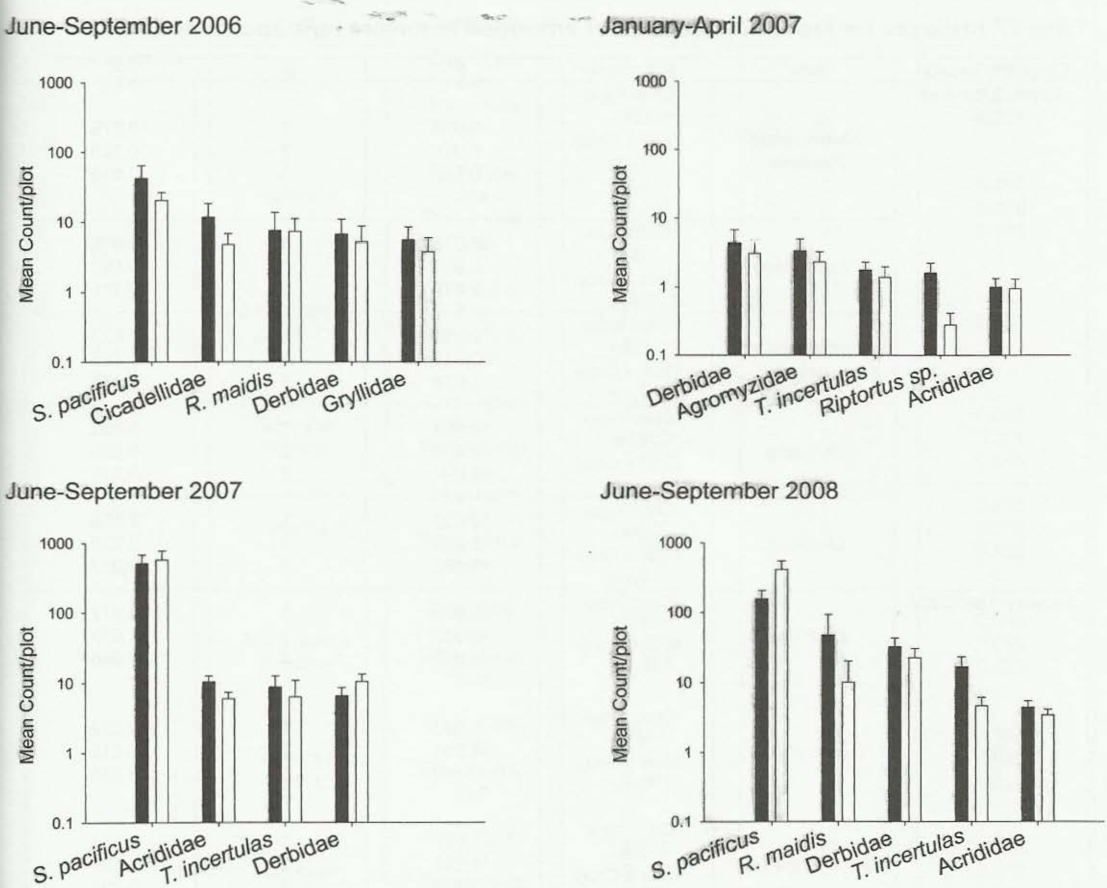
distribution was skewed in June-September 2006, June-September 2007 and June-September 2008 cropping seasons because of the significantly higher number of the corn leaf hopper *Stenocranus pacificus* Kirkaldy in June-September in non-Bt corn farm (Table 2). Other herbivores present in corn were the pink stem borer *Sesamia inferens* (Walker)(January-April 2007 to January-April 2009), corn leaf aphid *Rhopalosiphum maidis* (Fitch) (June-September 2006 and June-September 2008), Acrididae (January-April 2007 to January-April 2009), Agromyzidae (January-April 2007), Gryllidae (June-September 2006), Cicadellidae (June-September 2006 and January-April 2009) and the leaf bug *Riptortus* sp. (January-April 2007).

Influence of Crop Type on Natural Enemy Association. RDA analysis of abundance data did not reveal a clear trend in preferential association for a particular crop type (i.e. Bt or non-Bt) by natural enemies observed in corn (Figure 4). A total of ten natural enemy taxa were observed on both crop types in the entire duration of the study. The important seasonal natural enemies associated with Bt corn were *Micraspis discolor* (Fab.) (June-September 2006 and January-April 2007), *Proreus* sp. (Dermaptera, June-September 2007), fire ants (*Solenopsis geminata* , June-September 2008) and *Orius tantillus* Motschulsky (January-April 2009). The important natural enemies associated with non-Bt corn were lacewings (Chrysopidae, June-September 2006), spiders (Araneae, January-April 2007), unidentified wasp (June-September 2007), and *M. discolor* (June-September 2008 and January-April 2009).

Pest succession. Figure 5 shows the results of correspondence analysis of herbivore abundance data from each cropping season. The left panel indicates ordination in the space defined by the first two axes. The right panel of the figure summarizes successional patterns (Arpaia et al. 2007). In June-September 2006, samples were plotted in space defined by the first two axes, which explained 34.4% and 51.5% of the total variance, respectively. There is no clear separation between the treatments. The successional pattern in species assemblages clearly shows similar trends for Bt and non-Bt corn fields. Similar results were observed for the rest of the data also shown in Figure 5.

DISCUSSION

Numerous studies have been carried out to investigate the presence of risk of transgenic crops to arthropod natural enemies. In the present study, investigation was focused on the comparison of insect herbivore communities in Bt and non-Bt corn fields. Insect herbivores are in the forefront of studies on the effect of different ecological environments on speciation (Matsubayashi et al. 2010). To the best of our knowledge, this is the first long term monitoring study conducted in the Philippines to evaluate the ecological impact of Bt corn on herbivore community in a commercial production setting. The paired farmers' field design replicated three times over 4 years was used because it was appropriate for Tier 4 studies (Perry 2009) and sufficient to detect a 50% difference in effect of size. In the present study, a 62% mean difference in *S. pacificus* abundance was detected as statistically significant in favor of non-Bt corn. A 50% difference in arthropod abundance is considered ecologically significant and practical for detection in field studies (Blumel et al. 2000; Perry et al. 2003).



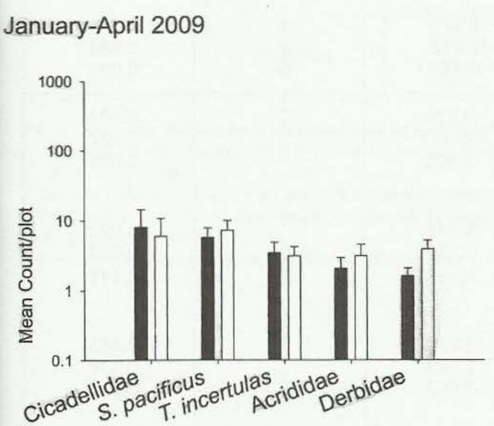


Figure 3. Rank abundance plot of herbivores in corn sites. Visual samplings of arthropods were conducted from June 2006 to April 2009. Effect of crop type, i.e. Bt (■) and non-Bt (□) was significant (P = 0.045) for *S. pacificus* in 2008 as determined by repeated measures ANOVA.

Table 2. Statistics for repeated measures of arthropod herbivore rank abundance data[a]

Cropping Season	Taxa[b]	Parameter	F[c]	df[d]	P[e]
June-September 2006	*Stenocranus pacificus*	Crop Type	0.030	1	0.878
		Site	6.970	2	0.125
		Time x Crop Type	0.902	5	0.516
	Cicadellidae	Crop type	0.018	1	0.906
		Site	9.839	2	0.092
		Time x Crop Type	2.413	5	0.111
	Rhopalosiphum maidis	Crop type	0.331	1	0.623
		Site	0.380	2	0.725
		Time x Crop Type	1.878	5	0.185
	Derbidae	Crop Type	0.307	1	0.635
		Site	0.600	2	0.625
		Time x Crop Type	3.297	1	0.211
	Gryllidae	Crop Type	0.037	1	0.865
		Site	3.221	2	0.237
		Time x Crop Type	3.165	5	0.057
January-April 2007	Derbidae	Crop Type	0.002	1	0.972
		Site	0.532	2	0.653
		Time x Crop Type	0.082	4	0.986
	Agromyzidae	Crop Type	0.333	1	0.622
		Site	0.231	2	0.812
		Time x Crop Type	0.189	5	0.960
	Sesamia inferens	Crop Type	1.777	1	0.314
		Site	4.242	2	0.191
		Time x Crop Type	0.079	5	0.994
	Acrididae	Crop Type	0.072	1	0.814
		Site	0.712	2	0.584
		Time x Crop Type	0.716	5	0.626
June-September 2007	*Stenocranus pacificus*	Crop Type	0.375	1	0.603
		Site	35.417	2	0.027
		Time x Crop Type	3.105	5	0.060
	Acrididae	Crop Type	9.543	1	0.091
		Site	11.098	2	0.083
		Time x Crop Type	2.123	5	0.146
	Sesamia inferens	Crop Type	1.230	1	0.383
		Site	2.063	2	0.326
		Time x Crop Type	0.952	4	0.483
	Derbidae	Crop Type	1.319	1	0.370
		Site	0.278	2	0.782
		Time x Crop Type	9.173	3	0.012

Cropping Season	Taxa	Parameter	F	df	P
June-September 2008	Derbidae	Crop Type Site Time x Crop Type	0.430 0.252 2.595	1 2 4	0.579 0.799 0.117
	Acrididae	Crop Type Site Time x Crop Type	2.071 4.567 2.156	1 2 5	0.287 0.180 0.141
	Stenocranus pacificus	Crop Type Site Time x Crop Type	20.903 10.891 0.540	1 2 5	0.045 0.084 0.743
	Sesamia inferens	Crop Type Site Time x Crop Type	1.205 0.985 2.661	1 2 5	0.387 0.504 0.088
January-April 2009	Cicadellidae	Crop Type Site Time x Crop Type	0.221 2.520 0.887	1 2 2	0.685 0.284 0.480
	Stenocranus pacificus	Crop Type Site Time x Crop Type	0.523 19.383 0.522	1 2 4	0.545 0.049 0.723
	Sesamia inferens	Crop Type Site Time x Crop Type	0.000 0.561 0.904	1 2 5	0.998 0.641 0.515
	Acrididae	Crop Type Site Time x Crop Type	0.276 2.157 0.209	1 2 2	0.652 0.317 0.820
	Derbidae	Crop Type Site Time x Crop Type	1.116 2.005 1.587	1 2 4	0.402 0.333 0.268

[a] Statistics were derived from repeated measures ANOVA of log (x+1) transformed arthropod count data gathered from visual sampling of 100 corn plants from each farm in every replicate at 25, 35, 45, 55, 60 and 110 days after planting from June 2006-April 2009.

[b] Taxa represents > 1% of total abundance in each farm.

[c] F-statistic is the ratio of two sample variances. A large F value indicates a statistically significant P.

[d] df, degrees of freedom

[e] P = 0.05 indicates significant treatment difference at $\alpha = 0.05$.

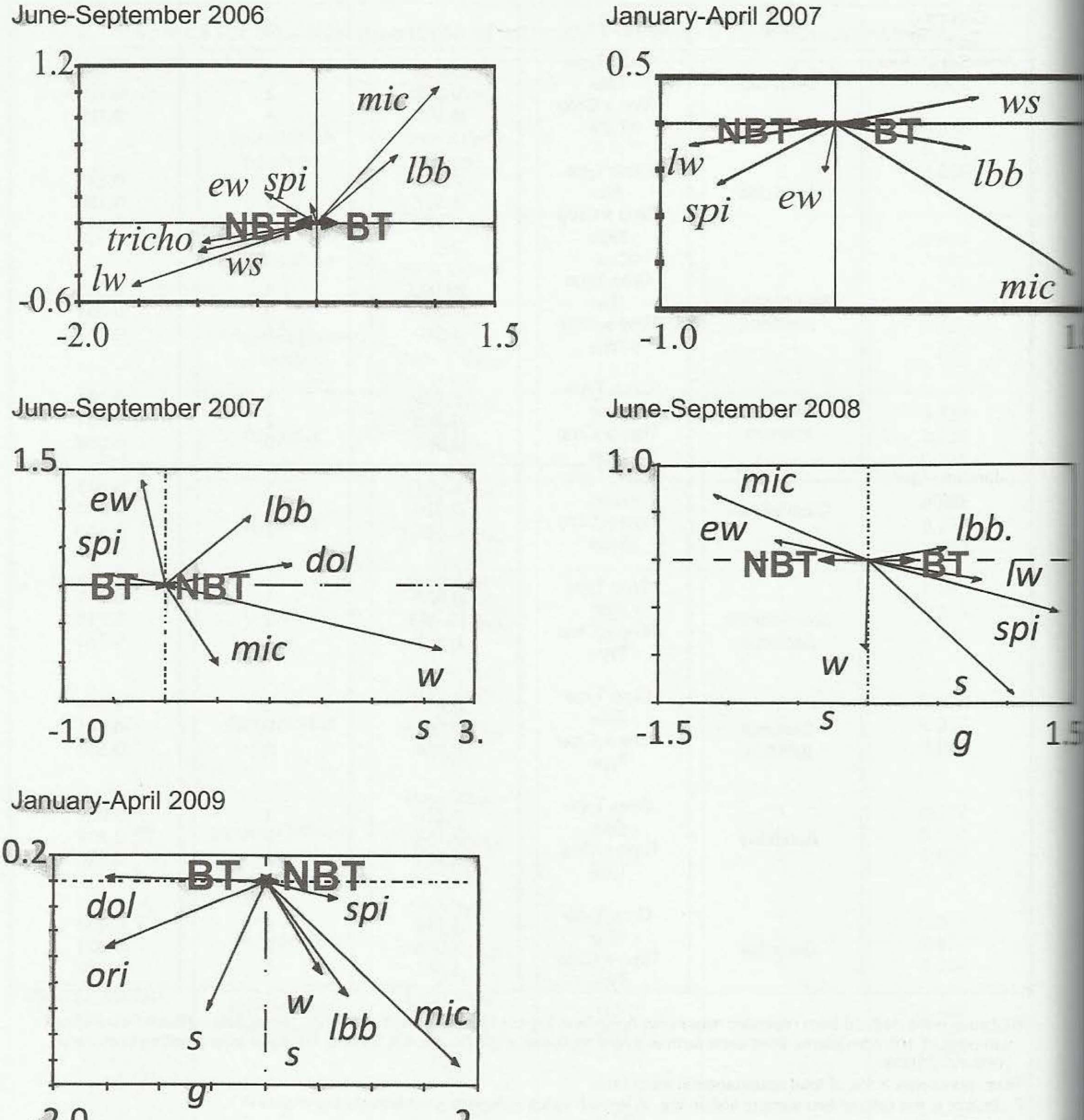


Figure 4. Biplots based on redundancy analyses of natural enemy abundance data gathered from visual samplings of three paired corn sites in Ocampo, Sañgay and Tigaon towns in Camarines Sur province. The horizontal and vertical axes represent sample scores and species weights which explain a fraction of the total variance of the data set. Arrows pointing roughly in the direction of crop type (i.e. Bt or non-Bt) indicate a high positive correlation. Length of arrow indicates degree of correlation of abundance of a taxon with a particular crop type. dol, DolichopodidaeHew, earwig (*Proreus* sp.)Hlbb, lady bird beetle (*Cheilomenes sexmaculatus*)Hw, lace wing (Chrysopidae)Hmic, *Micraspis discolor*+ori, *Orius tantillus*+sg, *Solenopsis geminata*+spi, spider (Araneae)Hricho; *Trichogramma* sp.Hws, wasp (I ymenoptera).

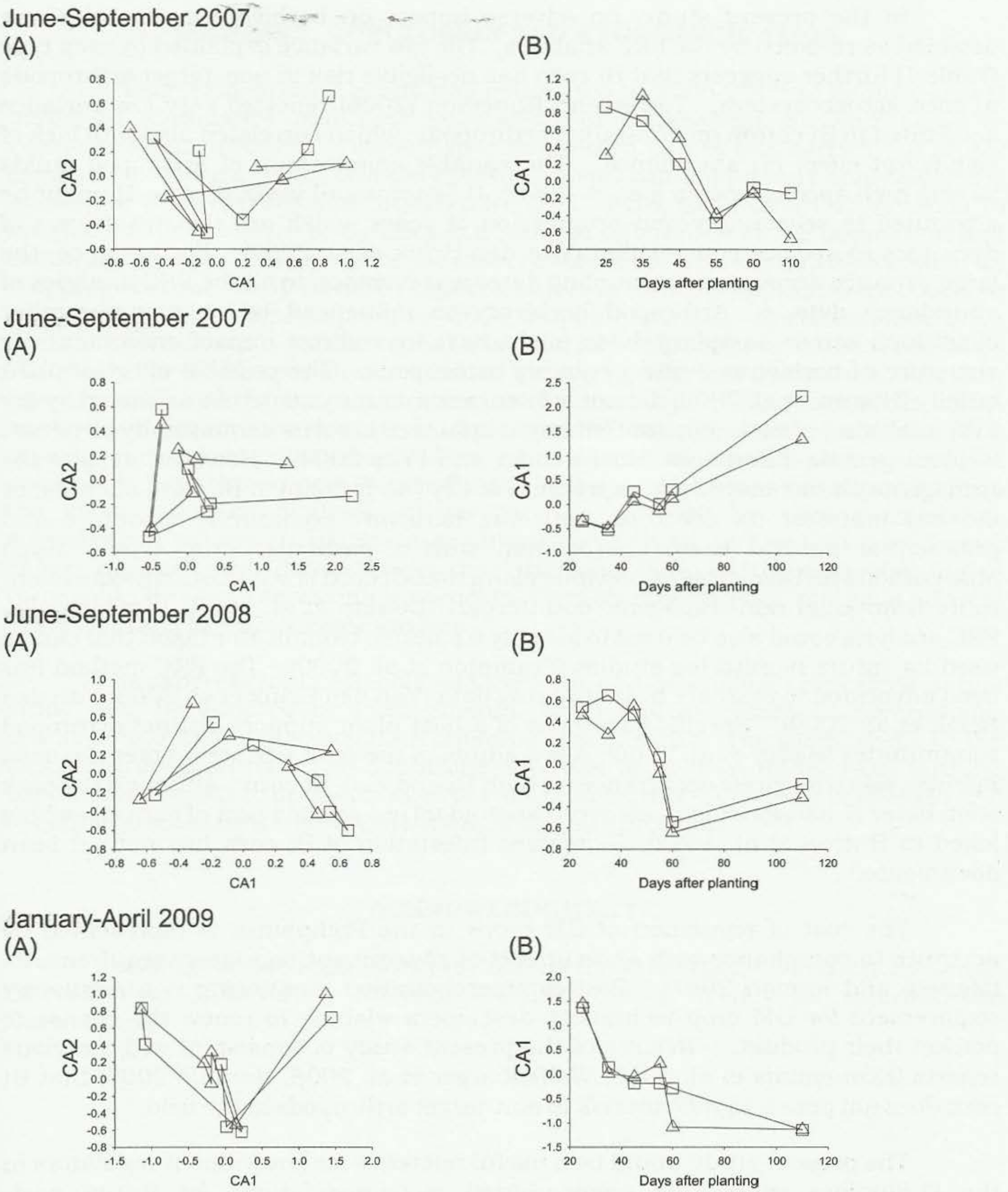


Figure 5. Correspondence analysis ordination of herbivore counts gathered from three paired corn sites in Ocampo, Sañgay and Tigaon towns in Camarines Sur province. Visual samplings of arthropods were conducted from June 2006 to April 2009. (A) Ordination of arthropod counts from Bt corn () and Non-Bt corn () in the space defined by the first two axes (CA1 and CA2). (B) Successional dynamics of species assemblage in Bt () and non-Bt () as summarized by CA1 scores. The first axis (CA1) is related to temporal changes in community structure. n both (A) and (B), a lack of clear separation between any two curves indicates insignificant treatment difference.

In the present study, no adverse impact on herbivore community was detected as revealed by the PRC analyses. The low variance explained by crop type (Table 1) further suggests that Bt corn has negligible risk to non-target arthropods in corn agroecosystem. Torres and Ruberson (2006) reported very low variance accounted to Bt cotton on non-target arthropods, which correlated also with lack of significant effect on abundance. The variable composition of arthropod guilds having high species scores (i.e., $\geq$ 0.5 or -0.5) across all years (Figure 1) might be attributed to seasonality and progression of years which are the two drivers of dynamics in species composition (Van den Brink et al. 2009). For instance, the large variance accounted to sampling dates was common to all the PRC analyses of abundance data. Arthropod herbivory as influenced by changing weather conditions across sampling dates might have an indirect impact on community structure of herbivorous and predatory arthropods. The possible effect of plant quality (Utsumi et al. 2009) did not influence community structure as shown by the PRC analysis. Another factor that might influence herbivore community structure is plant genetic differences (Hochwender and Fritz 2004). However, despite the transgenic characteristic (eg. expression of Cry1Ab protein) of Bt corn, such factor did not manifest its effect on both the herbivore community structure and preferential natural enemy association with a particular crop type. Such observations further support previous claim that Bt corn is substantially equivalent to its traditional non-transgenic counterpart (Donkin et al. 2003). Incidentally, PRC analysis could also be used to identify a suitable bioindicator taxon that can be used for future monitoring studies (Frampton et al. 2000). The PRC method has been advocated to evaluate biomonitoring data (Van den Brink et al. 2003, Van den Brink et al. 2009). Specific genotypes of a host plant support distinct arthropod communities (Bailey et al. 2006). A few adults of the pink rice stem borer *Sesamia inferens* was a surprise occurrence on both Bt and non-Bt corn. Although the pink stem borer *S. nonagrioides* (Lefebvre) (Noctuidae) is a serious pest of corn elsewhere (cited in Butron et al. 1999), *S. inferens* infestation of Bt corn has not yet been documented.

The cost of regulation of GM crops in the Philippines is represented by activities in compliance with and support of government regulatory requirements (Manalo and Ramon 2007). Post-commercialization monitoring is a regulatory requirement for GM crop technology developers wishing to renew the license to market their product. Results of the present study is consistent with previous reports (Economidis et al. 2010, Wolfenbarger et al. 2008, Naranjo 2009) that Bt corn does not pose a significant risk to non-target arthropods in the field.

The present study would be a useful reference for government regulators in the Philippines in making science-based recommendations for future post-commercialization monitoring of GM crops that are already in the regulatory pipeline, and in formulating policy guidelines for monitoring schemes, paving the way for coexistence with organic farming (Jones 2009).

SUMMARY, CONCLUSION AND RECOMMENDATION

A long term on-farm study was conducted in 2006-2009 to monitor insect herbivore abundance and pest succession in commercial Bt corn fields in Camarines Sur province. Visual inspection of corn plants was carried out on six separate dates during each cropping season. Insect count data were analyzed using univariate (RM-ANOVA) and multivariate (PRC, RDA, CA) statistical methods to analyze for the presence of significant treatment differences at the population and community levels. Derbids were the most common herbivore group observed in all cropping seasons. A significantly higher number of corn leaf hoppers were observed in non-Bt corn in June-September 2008 cropping season. Species composition within Bt corn farms is similar to that observed in non-Bt corn farms as revealed by PRC analysis. The low variance explained by crop type further suggests that Bt corn has negligible risk to non-target insect herbivores in corn agroecosystem. RDA analysis did not reveal a clear trend in preferential association for a particular crop type by natural enemies observed in corn. *Micraspis discolor* was a common predator in both Bt and non-Bt corn fields. Pest succession was not apparent from the correspondence analysis of the data as revealed by similar trends in successional pattern in species assemblages in both Bt and non-Bt corn farms. Taken together, the data showed that the long term commercial planting of transgenic Bt corn expressing insecticidal Cry1Ab protein does not have adverse effect on insect herbivore communities.

The experimental protocols and methods of data analyses described in this study may be adopted by local environmental risk assessors in government, academe and industry to evaluate potential risk of future transgenic crops to the local arthropod fauna. The continued adoption of Bt corn under local farming conditions remains to be encouraged to enhance arthropod diversity in the field and to help in promoting the government's food security program.

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