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NON-TARGET ORGANISMS ON Bt CORN MON89034 AND MON89034/NK603: PART I. ABUNDANCE AND DIVERSITY OF CANOPY- AND GROUND SURFACE-DWELLING ARTHROPODS IN REGULATED FIELD TRIAL SITES DURING DRY SEASON IN LUZON AND MINDANAO, PHILIPPINES[1]

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ABSTRACT

The effects of two new transgenic corn hybrids, MON89034/NK603 and MON89034, on the communities of non-target organisms (NTOs), specifically arthropods were studied during the dry season from January to May 2009. The regulated field trial sites were located in Luzon (Ilagan, Isabela and Sta. Maria, Pangasinan) and Mindanao (Tupi,South Cotabato). The test materials consisted of DK818 corn hybrid with MON89034 and MON89034/NK603 traits, and its unprotected and insecticide-protected conventional forms. Abundance (based on total counts) and species diversity were assessed across four test materials in three replicates arranged in a randomized complete block design. Arthropods were sampled with sweep net, net-bagging/vacuum sampling, pitfall trapping, sugar- and protein-baiting, and visual counts. Results revealed that treatments did not differ in terms of presence or absence of NTOs. The highest total number of NTOs was observed in Isabela while the lowest was in South Cotabato. Abundance and diversity for both canopy- and ground surface-dwelling NTOs did not differ significantly across treatments and sites. Among sampling periods,

abundance of canopy-dwellers were not significantly different at 25 and 60 DAP but had significant difference at 85 DAP. However, the differences among treatments were not consistent across sites. Abundance of ground surface-dwellers did not differ significantly across sites and sampling dates. Based on Shannon diversity index (H), the site in South Cotabato had the most diverse NTOs. Diversity indices among canopy- and ground surface-dwellers were not significantly different across treatments and sites, except in Pangasinan wherein MON89034/NK603 plots showed the highest diversity. Overall, there were no clear statistically significant differences obtained among the treatments across sites in terms of abundance and diversity. Hence, it can be concluded that based on the data gathered, the new transgenic traits have no significant positive or negative effects on non-target organisms. However, this has to be corroborated with an assessment of possible effects on functional guilds.

Key words: stacked Bt corn hybrid, regulated field trial, non-target organism, Asian corn borer, *Ostrinia furnacalis*, abundance, diversity

INTRODUCTION

Bt-corn has proven to be effective in keeping populations of the Asian corn borer, *Ostrinia furnacalis* (Guenee), below economically damaging levels. In the Philippines, this is best exemplified by Yieldgard™ (MON810) corn hybrid that has not shown signs of insect resistance development since its introduction in 2003. As an offshoot of this success, new products with broader targets have been developed and aimed for introduction to corn farmers in the country. These include MON89034 and MON89034/NK603. MON89034 contains two Bt proteins (cry1A.105 and cry2Ab2) and MON89034/NK603 is a stacked corn hybrid with the two aforementioned novel insecticidal proteins and another protein providing plant tolerance to the herbicide glyphosate. These second generation insect-protected corn varieties provide wider range of control against lepidopteran insect pests and an effective tool for insect resistance management. As required by biosafety regulations, environmental impacts of novel agricultural technologies including the introduction and use of modern biotechnological products like genetically modified organisms such as the two aforementioned corn varieties, need to be assessed. In the agroecosystem, the biodiversity profile is already a requirement in any study that aims to assess environmental impacts. In fact, biodiversity assessment is regarded as most important, considering the various threats to the different kinds of ecosystems and the environment in general.

Studies comparing Bt and non-Bt corn hybrids were conducted by Reyes & Jovillano-Mostoles (2005) and Reyes (2005). These were among the first of such studies under humid tropical conditions and provided valuable insights on the future of Bt-corn under Philippine conditions. However, aside from other shortcomings that were beyond the said researchers' control, they also utilized only sweep net sampling. This sampling method focuses mainly on species that are canopy-dwelling and which are largely diurnal (active during the day). Realizing that biodiversity is broad, it is therefore imperative to take into account also the other equally important arthropod components of the corn agroecosystem which may be flightless, nocturnal and not necessarily canopy-dwelling but which directly or indirectly affect the corn plant as herbivores or higher-order consumers. It is also noteworthy that Reyes & Jovillano-Mostoles (2005) likewise said that long-term studies are necessary to further characterize subtle population changes in the corn field as a result of the introduction and continuous utilization of Bt-corn. In Camarines Sur, Philippines, a study to monitor the abundance of herbivores and pest succession in commercial corn fields was conducted for 5 consecutive seasons (from June 2006 to April 2009) and results revealed that long term planting of Bt corn does not have harmful effects on non-target arthropods (Alcantara et al. 2010).

This study aimed to 1) determine the abundance of canopy- and ground surface-dwelling arthropods on Bt-corn MON89034 and MON89034/NK603 in confined field trials in Luzon (Isabela and Pangasinan) and Mindanao (South Cotabato); 2) classify the non-target arthropods according to their functional guilds; and 3) compare the abundance and diversity of these NTOs between Bt and non-Bt corn hybrids.

MATERIALS AND METHODS

Experimental Plots

Four (4) corn hybrids (treatments) were planted in regulated field trial sites in Luzon (Ilagan, Isabela and Sta. Maria, Pangasinan) and Mindanao (Tupi, South Cotabato) with three replicates each during the dry season from January to May 2009: DK818 MON89034/NK603 (T1- Bt corn hybrid), DK818 MON89034 (T2- Bt corn hybrid), DK818 Conventional (T3- Non-Bt corn hybrid) and DK818 Conventional with chemical protection (T4- Non-Bt corn) i.e. carbofuran at 25-30 days after planting (DAP) and cypermethrin at 45-50 DAP. Treatments 1, 2 and 3 had no chemical input or protection. Only hand weeding was applied to all plots when necessary.

The hybrids were planted in the confined trial sites following a planting distance of 0.75-0.80 m between rows and 20 cm between hills. The plots were laid following the Randomized Complete Block Design (RCBD) with each treatment replicated three times. Each plot had 10 rows, each 10 m long, which included two border rows plus two pollinator rows of the conventional hybrids. The surrounding area of the confined trial was planted with 5 m long rows of non-Bt conventional hybrid field corn to minimize potential edge effects. All the necessary cultural management practices in corn growing were employed. The national biosafety guidelines for regulated materials (Philippine Department of Agriculture Administrative Order (DAO) no. 8, series of 2002) were strictly followed through the regulatory body, the Bureau of Plant Industry of the Department of Agriculture.

Collection of Arthropods

Non-target organisms (NTOs) from eight (8) inner rows of corn in each treatment were collected using the different sampling methods taken at 25 (early vegetative), 60 (silking) and 85 (pre-harvest) days after planting. References were consulted for appropriate sampling times and techniques (Marshall et al. 1994, Naranjo et al. 2005, Stiling 2004).

Sweep net. This method collected mainly flying and canopy-dwelling arthropods. With the use of insect net, 20 sweeps were done in between the two test rows from the bottom (about one foot above the ground) up to about a meter above the canopy, ensuring that the leaves were not destroyed while sweeping. The collected specimens from each plot were placed immediately and sealed in a separate labeled plastic bag.

Visual count. Visual inspection was done on rows 2 and 6 of each plot. Each plant in the rows sampled was carefully examined from base up to topmost foliage (during vegetative stage) and up to the tassels (during silking and pre-harvest stages) and all arthropods observed were listed down in field notebooks. Opportunistic sampling was done to identify insects which cannot be identified in the field. This sampling is based on unique occurrences such as opportunities to capture or observe rare, uncommon or extraordinary species or forms. Collected samples were placed in microcentrifuge filled with 95% alcohol, labeled properly and brought to the laboratory for identification.

Bagging/vacuum sampling. Five (5) plants per row were randomly selected. During the early vegetative stage, a net or plastic bag was placed on each whole plant and carefully shaken to collect all terrestrial arthropods trapped within. This sampling was done immediately after the sweeping method. At the silking and pre-harvest stages, an improvised car battery-operated vacuum sampler was used as the plant was too large already to be enclosed in a net or plastic bag. All vacuum collected arthropods were likewise placed in separate labeled plastic bags.

Pitfall trapping. Plastic cups (approx. 250 ml capacity) were used as pitfall traps with lid. Two cups, one on top of the other, were securely placed in a hole dug to the level of the lid of the lower cup (ca. 12.5 cm). Afterwards the top cup into which soil particles accumulated are removed, leaving the clean bottom cup. Preservative fluid was then poured into the clean cup up to around one-fourth full. Dried leaves or similar material were used to conceal the pitfall before its cover was placed immediately above attached to a barbecue stick to minimize chance of flooding in case of rain but left ample space for prospective catches to fall in. As the study targeted mainly aboveground arthropods, only groups or species that are known or observed to forage on the plant canopy were considered among the pitfall trap catches and the rest which are strictly ground surface-dwelling (e.g. collembolans) were set aside.

Baiting. Three (3) baits, each consisting of layers of rectangular folded white tissue paper (ca. 5 cm x 10 cm) moistened with concentrated sugar solution, were placed under a corn plant in between two adjacent rows. Baits were set randomly and left undisturbed for an hour to allow ants to gather on them. Another set of similarly sized baits but using half teaspoon of tuna flakes, together with its vegetable oil instead of sugar solution, was exposed to attract carnivorous ants and left also for at least an hour before collection. Each bait material was collected using forceps and placed immediately in individual plastic bags and sealed, taking care to capture as many of the gathered ants as possible. Ethanol was then poured through a wash bottle to kill the ants.

Sorting, Preservation and Identification of Collected Specimens

The standard methods for sorting and preserving were followed and done in the laboratory. Sorting was done under a dissecting microscope or good magnifying lense for relatively larger specimens.

In general, immature stages and soft-bodied arthropods were preserved in 95% ethyl alcohol. Ants were killed and preserved also in ethanol but representatives for each species per batch were mounted on pinned card points. Mites, aphids and other small insects, as well as portions of insects that needed to be examined under a compound microscope, were mounted on glass slides, usually as temporary mounts using modified Hoyer's medium (formula: chloral hydrate, gum Arabic, glycerine, distilled water). Prior to mounting, most specimens were macerated with 5-10% KOH or NaOH, dehydrated through an alcohol series and cleared (for fatty specimens, a 1:1 mixture of phenol and xylene). Prepared slides were dried in an oven or at room temperature for at least a week and then cleaned and sealed with colorless nail polish. Larger specimens were mounted on insect pins. For all cases, standard data labels were attached to each specimen, either on the glass slide, to the insect pin or placed in the vial with liquid preservative.

Identification was done using pertinent literature or in consultation with available local specialists, in cases of arthropods that were beyond the expertise of the project leader and staff. Specimens were identified to species level whenever possible for the common groups or at least up to genus level. Those belonging to less taxonomically known groups were identified to the family level. The number of individuals per species was counted. For those that could not be identified to genus or species level, each morphologically similar cluster or group (morphospecies) was assumed to constitute a single species. A hand tally counter was used for species with more than 20 individuals.

In classifying the arthropods into functional guilds, groupings were generally based both on feeding habits observed during fieldwork and information in published literature. Inferring or extrapolating was done especially for species collected or trapped but not directly observed feeding. The non-target functional guilds included herbivores, predators, parasitoids, neutrals and pollinators. Herbivores ("pests" other than the Asian corn borer, earworm and common cutworm) on corn were subdivided into chewing and sucking insects. The neutrals consisted of scavengers, detritivores and vagrants and were lumped with pollinators in the total counts.

Digital Photodocumentation, Data Collation, Encoding and Analysis

Each activity, arthropod species, or the most ecologically significant among them, was photographed to document the presence and/or role of the organism in the corn agroecosystem.

All the data collected and observations recorded in the field and laboratory activities were collated, organized and encoded using Microsoft Excel. Included were names of orders, families, genera, species and number of individuals per species or morphospecies. The total number of individuals and species belonging to each of the non-target guilds, namely, non-target herbivores, predators, parasites/parasitoids were computed for each treatment. Indices of arthropod diversity (Shannon index (H), evenness, dominance etc.) were computed using BioDiversity Professional Beta 1 Version. Statistical analysis to compare treatment differences was done using SPSS v13.

Voucher Specimen Deposition

Voucher specimens were deposited in the Entomology Section of the UPLB Museum of Natural History, both in compliance with the provisions of wildlife protection law and in adherence to accepted standards for studies involving bioprospecting and biodiversity.

RESULTS AND DISCUSSION

Abundance of Non-Target Arthropods

Canopy-dwelling. The abundance of canopy-dwelling non-target arthropods (NTOs) on MON89034 (T2) and MON89034/NK603 (T3) did not differ significantly from those on the conventional and chemically protected treatments in Isabela, Pangasinan and South Cotabato throughout all sampling periods i.e. 25, 60 and 85 DAP (Figure 1). However, MON89034 (T2) had numerically more NTOs in Isabela compared to other treatments.

Considering the total number of individuals during each sampling period, the differences among the treatments were also not significant in all the three regulated field sites at 25 and 60 days after planting (DAP) (Figure 2). At 85 DAP, significant differences were observed in Isabela, Pangasinan and South Cotabato. However, the trend in responses of NTOs to each treatment was not consistent. Specifically, in Isabela, the conventional hybrid (T3) had significantly fewer NTOs compared to both MON89034 (T2) and the chemically protected treatment (T4), but not with MON89034/NK603 (T1). In turn, MON89034/NK603 (T1) did not differ significantly from MON89034 (T2) and chemically protected (T4) corn plants. In Pangasinan, MON89034/NK603 (T1) and chemical protection (T4) differed significantly from each other whereas both MON89034 (T2) and the conventional hybrid (T3) were intermediate and did not differ significantly from either of those two. In contrast, MON89034/NK603 (T1) had significantly more NTOs than MON89034 (T2) whereas the conventional hybrid (T3) and chemically protected (T4) were intermediate. Even among the 25 and 60 DAP results that were not significantly different, the responses of NTOs to the treatments were also not consistent in all sites.

Ground surface-dwelling. The treatments did not differ significantly in all the regulated field sites in total number of non-target ground surface-dwelling arthropods considering the entire season totals (Figure 3) and the three sampling periods (25, 60 and 85 DAP) (Figures 4). It is also worth noting that occurrence of the highest number of ground surface-dwelling arthropods was generally not consistent in

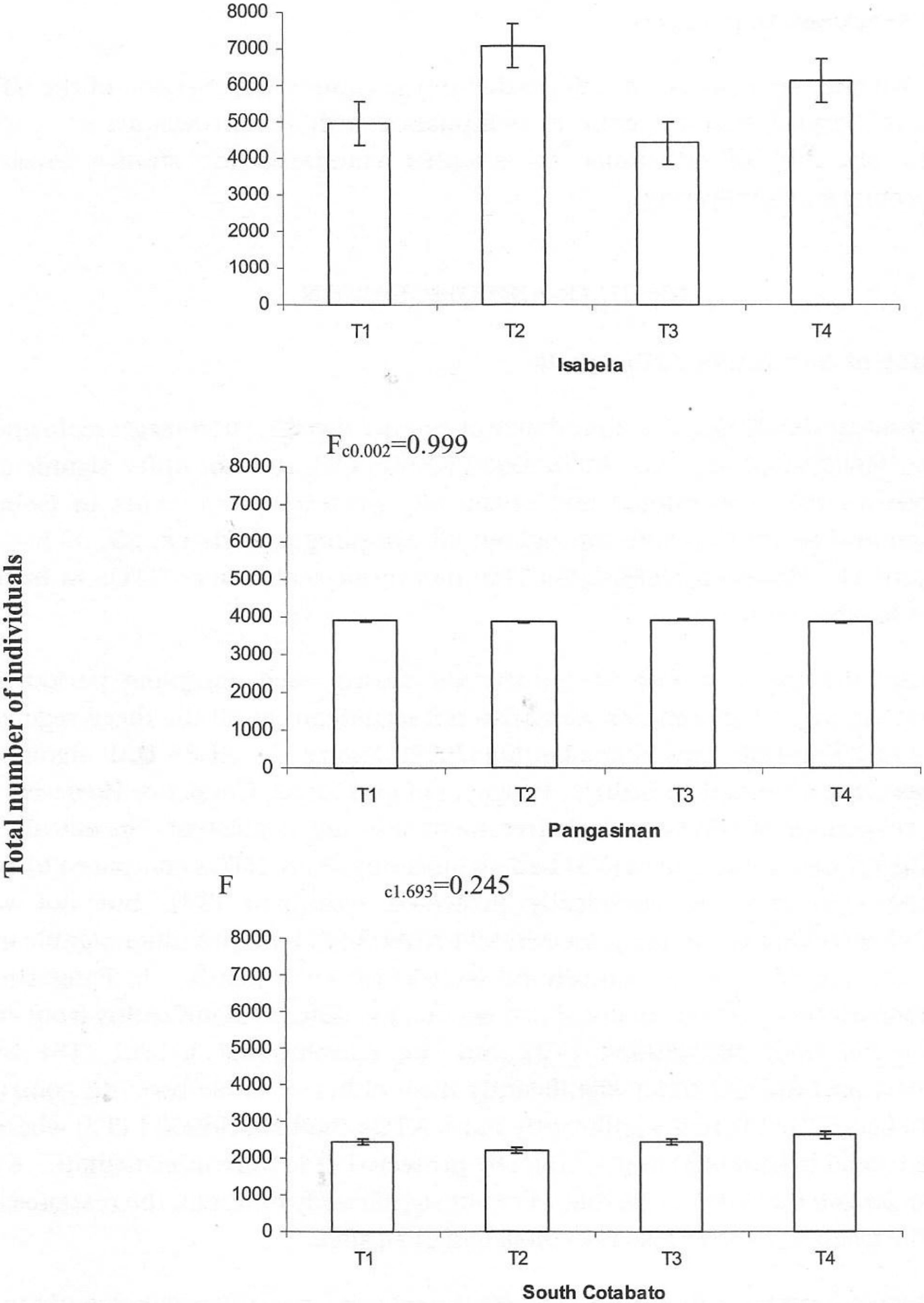

Figure 1. Comparative abundance of canopy-dwelling non-target arthropods for each treatment in the regulated field trial sites in Isabela, Pangasinan and South Cotabato. T1- Bt corn hybrid MON 89034/NK603; T2- Bt corn hybrid MON 89034; T3- Conventional corn hybrid; T4- Conventional corn hybrid with chemical protection. Totals are for all DAPs during the dry season 2009.

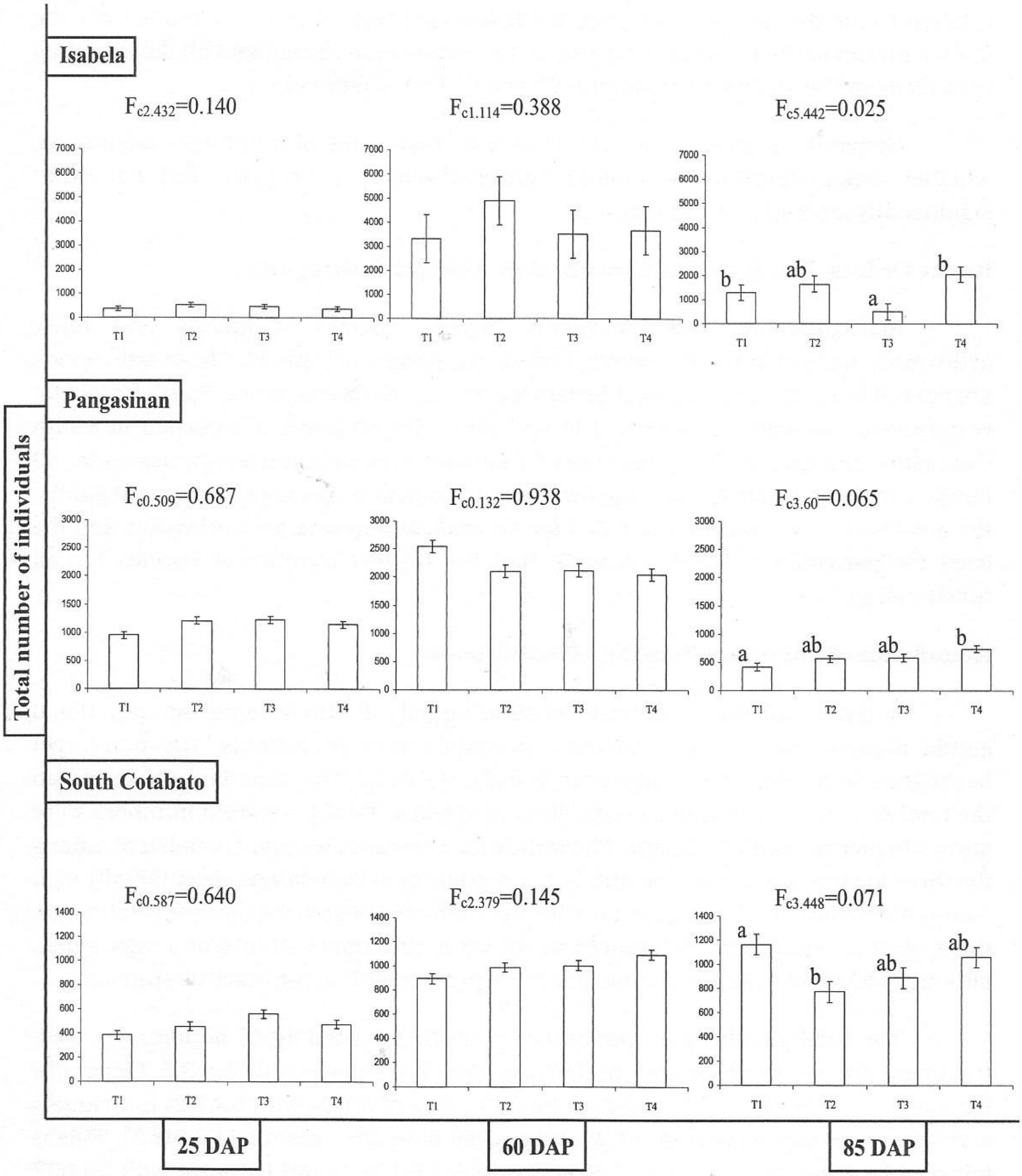

Figure 2. Comparative abundance of canopy-dwelling non-target arthropods at each sampling period (days after planting, DAP) for each treatment in the regulated field trial sites in Isabela, Pangasinan and South Cotabato. T1- Bt corn hybrid MON 89034/NK603; T2- Bt corn hybrid MON 89034; T3- Conventional corn hybrid; T4- Conventional corn hybrid with chemical protection.

relation to the treatments. However, the insecticide-treated plots tended to have the fewest ground surface-dwelling arthropods in Pangasinan throughout all the sampling periods as well as in South Cotabato at 25 and 60 DAP (Figure 4).

Generally, therefore, the occurrence or responses of non-target organisms, whether canopy-dwelling or ground surface-dwelling arthropods, did not differ significantly among the treatments.

Insect Orders, Families and Species of Non-Target Arthropods

Identification and incidence. Several species of insects and other arthropods were identified to several orders and families (Table 1). These arthropods are spread in 22, 23 and 24 insect orders for Isabela, Pangasinan and South Cotabato, respectively. Overall, there were 134 species under 84 families recorded in South Cotabato compared to 98 species under 67 families in Isabela and 92 species under 59 families in Pangasinan. Classifying these arthropods into ecological functional guilds, the greatest proportion was recorded for neutrals and non-target herbivores and the least for parasitoids. South Cotabato had the highest number of species for all functional guilds.

Abundance of Important Non-Target Arthropods

In terms of total abundance considering only the three important functional guilds namely, non-target herbivores, predators and parasitoids, the non-target herbivores were consistently higher in Isabela (Table 2). The abundance is based on the total number of individuals regardless of species. Total parasitoid numbers were always higher in South Cotabato. The trends for predators were not consistent among the three locations although the number of predators in Isabela was exceptionally high compared to those in Pangasinan and South Cotabato. Comparing the four treatments, there were no significant differences in the total abundance of all non-target guilds although MON89034 appears to harbor more predators than the other treatments.

The total numbers of individuals classified according to families for each treatment during the dry season in the three sites are shown in Tables 3-5. Generally, the site in Isabela showed the highest total number of NTOs with 63,732 individuals across three sampling periods at 25, 60 and 85 days after planting (Table 3). This is followed by Pangasinan with 39,565 individuals (Table 4) and the least with 25,637 individuals in South Cotabato (Table 5). Among the non-target herbivores, delphacids were the most dominant in all the three sites with Isabela having the highest counts followed by Pangasinan. These were mostly *Stenocranus pacificus* which infested the

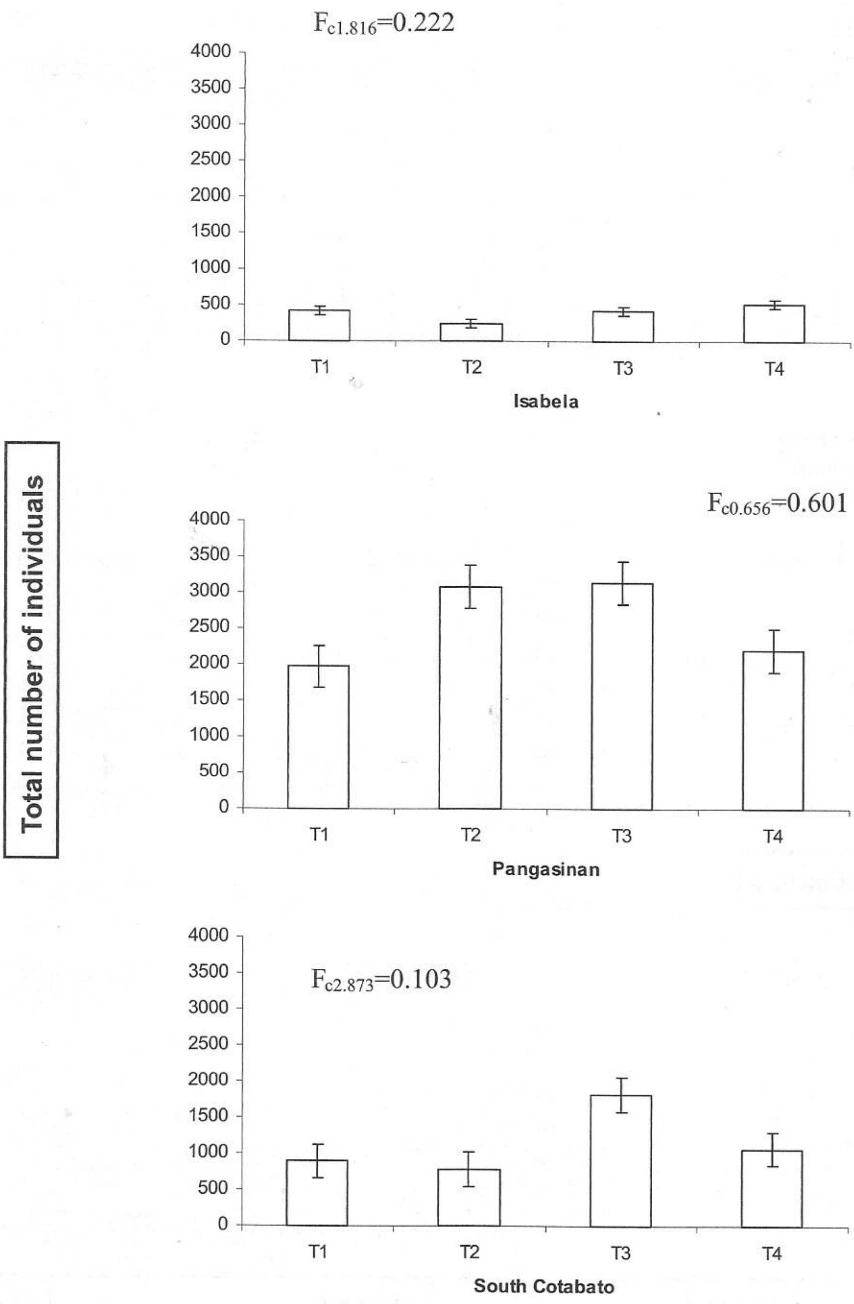

Figure 3. Comparative abundance of ground-dwelling arthropods for each treatment in the regulated field trial sites in Isabela, Pangasinan and South Cotabato. T1- Bt corn hybrid MON 89034/NK603; T2- Bt corn hybrid MON 89034; T3- Conventional corn hybrid; T4- Conventional corn hybrid with chemical protection. Totals are for all DAPs throughout the dry season 2009.

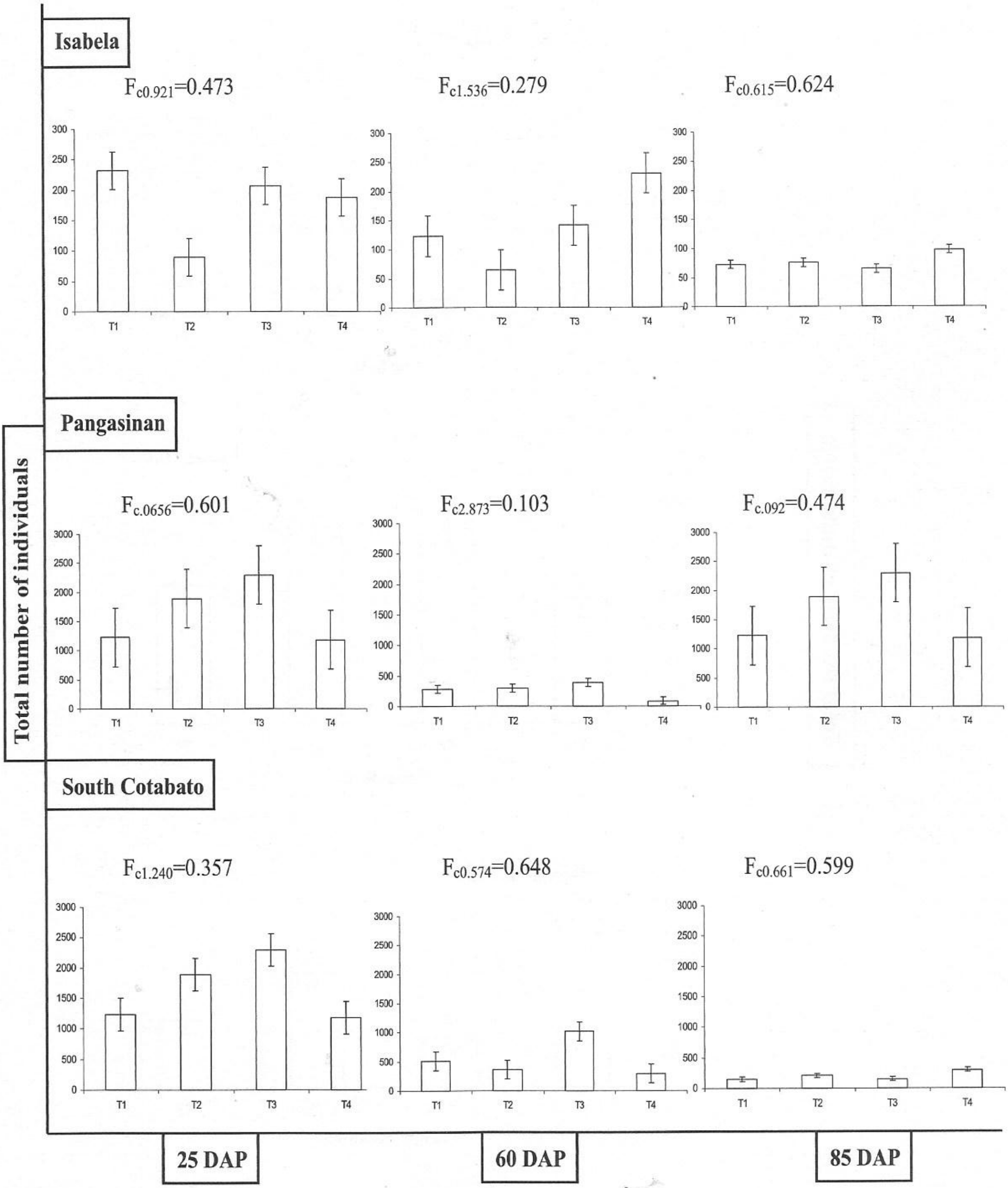

Figure 4. Comparative abundance of ground-dwelling arthropods at each sampling period (days after planting, DAP) for each treatment in the regulated field trial sites in Isabela, Pangasinan and South Cotabato. T1- Bt corn hybrid MON 89034/NK603; T2- Bt corn hybrid MON 89034; T3- Conventional corn hybrid; T4- Conventional corn hybrid with chemical protection.

Table 1. Number of orders, families and species of non-target arthropods under each functional guild collected and/or observed on *Bt*-corn (MON89034/NK603 and MON89034) and non-Bt conventional corn hybrids in the regulated field trial sites in Luzon and Mindanao during the dry season cropping (January-May 2009).

Order/Family/Species	Isabela	Pangasinan	South Cotabato
HERBIVORES (CHEWING)			
Order	4	4	4
Family	15	8	15
Species	22	12	28
HERBIVORES (SUCKING)			
Order	2	1	1
Family	8	7	7
Species	11	9	12
PREDATORS			
Order	9	9	9
Family	19	21	23
Species	38	35	41
PARASITOIDS			
Order	2	2	2
Family	5	5	8
Species	10	9	12
NEUTRALS			
Order	5	7	8
Family	20	18	31
Species	27	27	41
OVER-ALL			
Order	**22**	**23**	**24**
Family	**67**	**59**	**84**
Species	**98**	**92**	**134**

Table 2. Total number of individuals of non-target herbivores, predators and parasitoids collected from Bt and non-Bt corn hybrids in the regulated field trial sites in Isabela, Pangasinan and South Cotabato during the dry season cropping, January-May 2009 (Numbers are pooled totals for the three sampling methods: visual count, net bagging and sweep net).

GUILD/TRIAL SITES	MON89034/NK603 (T1 Bt corn)	MON89034 (T2 Bt corn)	Conventional (T3 Non-Bt corn)	Conventional with chemical protection (T4 Non-Bt corn)	TOTAL
Non-target herbivores (pests)					
Isabela	12247	15903	10693	15133	53976
Pangasinan	8839	8199	8097	7873	33008
South Cotabato	5004	4464	4460	5295	19223
Subtotal	**26,090**	**28,566**	**23,250**	**28,301**	**106,207**
Predators					
Isabela	1686	4158	1491	2376	9711
Pangasinan	1623	1562	1629	1717	6531
South Cotabato	1861	1236	1584	1635	6316
Subtotal	**5,170**	**6,956**	**4,704**	**5,728**	**22,558**
Parasitoids					
Isabela	8	9	16	12	45
Pangasinan	7	2	8	9	26
South Cotabato	34	20	17	27	98
Subtotal	**49**	**31**	**41**	**48**	**169**
GRAND TOTAL	**31,309**	**35,553**	**27,995**	**34,077**	**128,934**

Table 3. Total number of individuals of non-target herbivores, predators and parasitoids collected from Bt and non-Bt corn hybrids in the regulated field trial sites in Isabela during the dry season cropping, January-May 2009 (Numbers are pooled totals for the three sampling methods: visual count, net bagging and sweep net).

ISABELA	MON89034/NK603 (T1 Bt corn)	MON89034 (T2 Bt corn)	Conventional (T3 Non-Bt corn)	Conventional with chemical protection (T4 Non-Bt corn)	TOTAL
Non-target herbivores (pests)					
Delphacidae	11745	15190	10275	14661	51871
Cicadellidae	332	283	253	280	1148
Pyralidae	34	33	27	19	113
Chrysomelidae	102	160	121	103	486
Derbidae	34	237	17	70	358
Subtotal	**12,247**	**15,903**	**10,693**	**15,133**	**53,976**
Predators					
Spiders	532	715	370	677	2294
Formicidae	8	1436	29	63	1536
Coccinellidae	198	263	232	338	1031
Anthocoridae	146	549	194	123	1012
Miridae	690	1086	541	706	3023
Chrysopidae	112	109	125	469	815
Subtotal	**1,686**	**4,158**	**1,491**	**2,376**	**9,711**
Parasitoids					
Trichogrammatidae	5	3	4	4	16
Braconidae	3	6	12	8	29
Subtotal	**8**	**9**	**16**	**12**	**45**
GRAND TOTAL	**13,941**	**20,070**	**12,200**	**17,521**	**63,732**

Table 4. Total number of individuals of non-target herbivores, predators and parasitoids collected from Bt and non-Bt corn hybrids in the regulated field trial sites in Pangasinan during the dry season cropping, January-May 2009 (Numbers are pooled totals for the three sampling methods: visual count, net bagging and sweep net).

PANGASINAN	MON89034/NK603 (T1 Bt corn)	MON89034 (T2 Bt corn)	Conventional (T3 Non-Bt corn)	Conventional with chemical protection (T4 Non-Bt corn)	TOTAL
Non-target herbivores (pests)					
Delphacidae	8572	7972	7720	7599	31863
Cicadellidae	245	183	341	245	1014
Chrysomelidae	11	18	18	19	66
Derbidae	6	10	6	7	29
Pyralidae	5	16	12	3	36
Subtotal	**8,839**	**8,199**	**8,097**	**7,873**	**33,008**
Predators					
Spider	420	522	536	525	2003
Coccinellidae	513	463	459	585	2020
Formicidae	53	67	78	80	278
Miridae	543	412	468	435	1858
Chrysopidae	14	17	16	13	60
Anthocoridae	80	81	72	79	312
Subtotal	**1,623**	**1,562**	**1,629**	**1,717**	**6,531**
Parasitoids					
Braconidae	7	2	8	8	25
Ichneumonidae	0	0	0	1	1
Subtotal	**7**	**2**	**8**	**9**	**26**
GRAND TOTAL	**10,469**	**9,763**	**9,734**	**9,599**	**39565**

Table 5. Total number of individuals of non-target herbivores, predators and parasitoids collected from Bt and non-Bt corn hybrids in the regulated field trial sites in South Cotabato during the dry season cropping, January-May 2009 (Numbers are pooled totals for the three sampling methods: visual count, net bagging and sweep net).

SOUTH COTABATO	MON89034/NK603 (T1 Bt corn)	MON89034 (T2 Bt corn)	Conventional (T3 Non-Bt corn)	Conventional with chemical protection (T4 Non-Bt corn)	TOTAL
Non-target herbivores (pests)					
Delphacidae	3105	3289	3831	3573	13798
Cicadellidae	156	163	187	187	693
Chrysomelidae	26	5	26	23	80
Derbidae	661	343	297	679	1980
Aphididae	1052	661	108	823	2644
Pyralidae	4	3	11	10	28
Subtotal	**5,004**	**4,464**	**4,460**	**5,295**	**19,223**
Predators					
Spider	122	142	117	121	502
Coccinellidae	190	207	266	190	853
Miridae	181	179	249	223	832
Anthocoridae	60	48	44	50	202
Formicidae	335	240	230	397	1202
Dermaptera	965	396	662	645	2668
Chrysopidae	8	24	16	9	57
Subtotal	**1,861**	**1,236**	**1,584**	**1,635**	**6,316**
Parasitoids					
Braconidae	26	8	7	20	61
Ichneumonidae	8	11	9	7	35
Trichogrammatidae	0	1	1	0	2
Subtotal	**34**	**20**	**17**	**27**	**98**
GRAND TOTAL	**6,899**	**5,720**	**6,061**	**6,957**	**25,637**

fields starting at late vegetative stage. Aphids were abundant only in South Cotabato. For predators, spiders especially derby spiders, were the most common in Isabela and Pangasinan followed by coccinellids (ladybird beetles) and mirids (predatory bugs). The dominant predators in South Cotabato were dermapterans (earwigs) followed by coccinellids. Among the different treatments, there was no consistent trend in the total number of individuals for these different functional groupings. Results similar to those of previous studies by Reyes (2002 and 2005), Reyes et al. (2005) and Alcantara et al. (2010) were obtained i.e., Bt corn harbored most predators and parasitoids in the field, most probably consequential to the absence of chemical insecticide treatment during the cropping season.

For the target pest, it has been observed that the Asian corn borer was abundant on conventional corn hybrids (T3 and T4) especially the one with chemical protection (T4). The plants were already brown and falling down due to intensive stalk boring of the ACB at 85 DAP. Both the Bt corn hybrids were, as expected, resistant to the target pests and they remained green and undamaged.

For other arthropods associated with corn, many of them were commonly encountered in the test fields, regardless of variety/cultivar or locality. The non-target herbivores included what may be presently or previously considered secondary pests of corn. Nonetheless, a few species were noticeably more common in Luzon (Isabela and Pangasinan) than in Mindanao (South Cotabato) and vice-versa. There were also uncommon species like mayflies and caddisflies in the field which were previously not reported. For comparison however, there are very limited references specifically describing the different arthropods under each functional guild associated with corn. The only available literature includes those of Gabriel (1971, 1997) and Capco (1959) which recorded only the pests of corn. For the natural enemies of the Asian corn borer, several references were available like those of Camarao (2003), Javier et al. (1993) and Alba (1988).

Diversity Indices

Diversity indices (Shannon, H) were also not significantly different among the treatments for both canopy-dwelling and ground surface-dwelling non-target arthropods (Figure 5). It was only in Pangasinan that MON89034/NK603 (T1) had significantly more diverse ground-dwellers than both MON89034 (T2) whereas the conventional hybrid (T3) and the chemically protected treatment (T4) were both intermediate (Figure 6).

The results showed that generally there were no significant differences among treatments and that there was no clear trend as to possible positive or negative effects of each treatment on the diversity of non-target organisms. Also, the responses of NTOs to each treatment were again not consistent throughout all the regulated field sites. Specifically, the non-target arthropods were most numerous in Ilagan, Isabela with the numbers dominated by a single species, the corn planthopper *Stenocranus pacificus* (Table 2). The numerous arthropods from Sta. Maria, Pangasinan were also dominated by the corn planthopper (Table 4). Among the three sites, the fewest arthropods in terms of number of individuals were observed in Tupi, South Cotabato (Table 5). The overwhelming numbers of CPH in Isabela and Pangasinan, therefore, proved that considering plain abundance or total number of NTOs alone maybe misleading or not reflective of the true picture of the NTO communities. Hence, abundance data should always be accompanied and complemented by diversity indices.

NTO Diversity in the Three Sites

Although the diversity values did not differ significantly among the treatments in all the three sites, in general, the canopy arthropod communities in Isabela and Pangasinan were less diverse than that in South Cotabato (Figure 5). The numerical values for both Isabela and Pangasinan, ranging from >0.4 to <0.65, were probably affected by the sheer dominance of a single species, *S. pacificus*, during the outbreak of the said insect. In contrast, the diversity indices among canopy-dwelling NTOs for South Cotabato were all higher than 1.0. For all three areas, the diversity indices of ground-dwellers were less than 1.0 and in fact, less than 0.7 for both Pangasinan and South Cotabato.

Previous studies that documented differences in the diversity of arthropod communities among various habitats, geographical areas and degrees of anthropogenic disturbance correlated arthropod diversity with vegetation cover (e.g. Knops et al., 1999; Perner et al., 2003, 2005). As the regulated field trials were quite uniform experimental corn fields, similar correlations would not be possible and thus, any possible differences in arthropod community diversity would probably be a function of the diversity of either the surrounding plant communities or of their associated arthropods or both. Hence, at the onset, documentation of the vegetation in the immediate surroundings of the regulated field trials was included and this will be presented in another paper. The possible influence of the surrounding vegetation on differences or similarities (or lack of them) among treatments was also considered especially when the general trend showed no significant differences among the treatments in terms of NTO abundance, diversity and the taxonomic groups or species

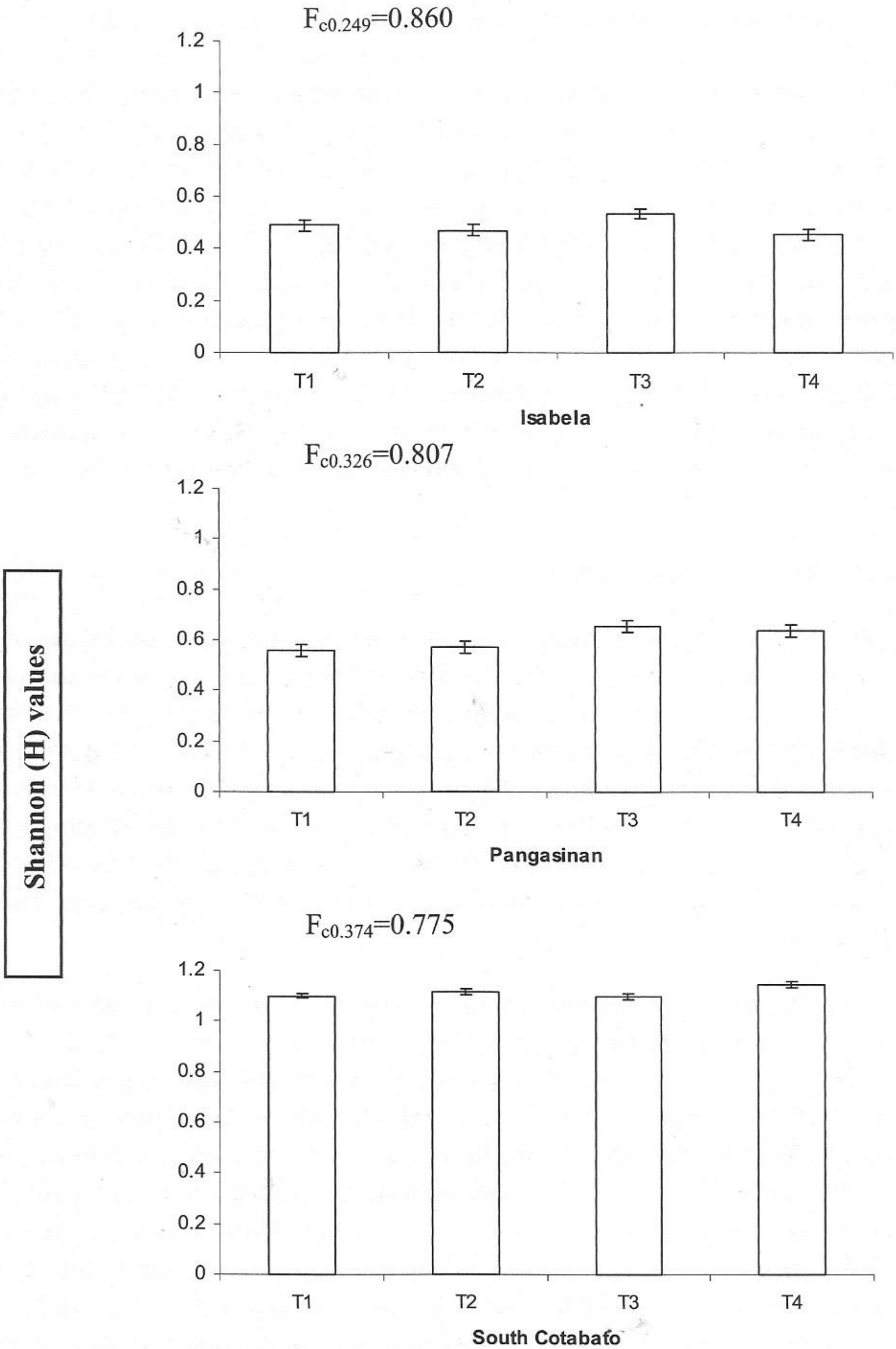

Figure 5. Diversity indices for canopy-dwelling arthropods for the different treatments in Isabela, Pangasinan and South Cotabato. T1- Bt corn hybrid MON 89034/NK603; T2- Bt corn hybrid MON 89034; T3- Conventional corn hybrid; T4- Conventional corn hybrid with chemical protection.

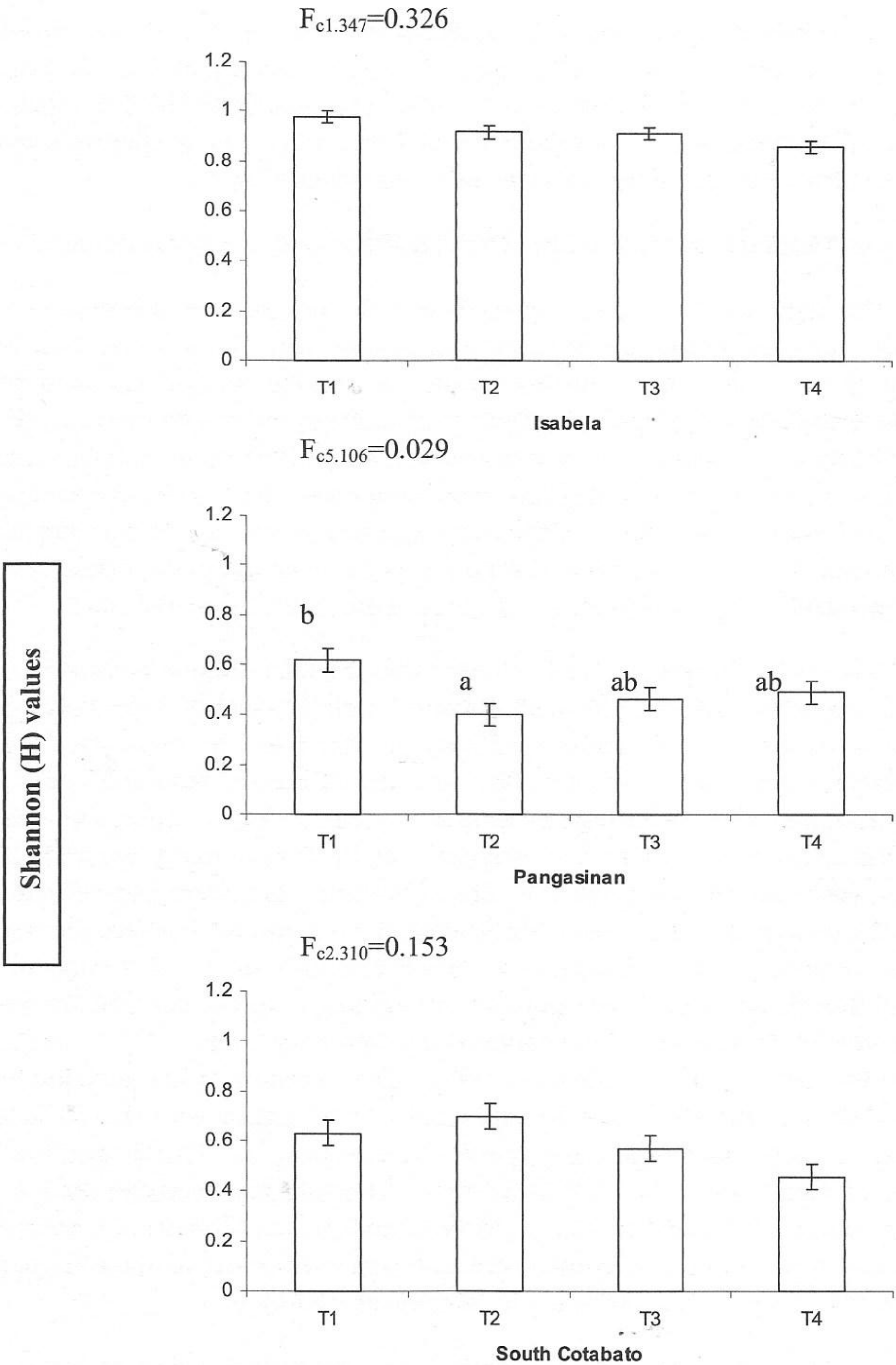

Figure 6. Diversity indices for the ground-dwelling arthropods for the different treatments in Isabela, Pangasinan and South Cotabato. T1- Bt corn hybrid MON 89034/NK603; T2- Bt corn hybrid MON 89034; T3- Conventional corn hybrid; T4- Conventional corn hybrid with chemical protection.

that were observed among them. In previous studies abroad, Bt-engineered plants generally harbored more predators and parasitoids as compared to the insecticide-treated or chemically protected plants. The lack of significant difference or this deviation from what is generally expected of either Bt plants or chemical protection could possibly be due to factors external to the corn field trials.

GENERAL TRENDS, INFERENCES, CONCLUSIONS AND RECOMMENDATION

The data obtained in this study reveal the occurrence of a diverse array of predators and parasitoids as well as herbivorous species ("pests") other than the target pests in all the treatments regardless of the corn variety and in regulated field trial sites. Nevertheless, actual numerical values of diversity indices showed that the site in South Cotabato had more diverse arthropod communities than in both Luzon sites (Isabela and Pangasinan). The observed incidence of various insects and other arthropods among the relatively diverse vegetation in the areas surrounding each regulated field trial site suggests that such vegetation cover plays important roles in the occurrence and abundance of non-target organisms inside the field trials.

The currently available data suggest that the introduction or presence of new genetically modified hybrids, MON89034 and MON89034/NK603, probably does not directly or indirectly affect either positively or negatively the non-target organisms particularly during the dry season. The existence of natural enemies, some of them notably abundant like the earwigs in South Cotabato, the derby spiders in Isabela and the ladybird beetles in all three sites, suggests that the surrounding vegetation provides habitats where natural enemies and other non-target organism can retreat to after chemical treatments of the insecticide-protected plots and from where they can come back to the corn plants to forage. Also, such existence in the field and the vicinity suggests that these natural pest population regulation agents continue to work and maintain most of the pests below economically damaging levels. With the exception of the CPH in northern Luzon, other pests were also in relatively low population levels. This presence of the CPH may be explained by the aggressive behavior and other inherent characteristics of invasive species in newly occupied areas and the general features of most insects being R-strategists. R-strategists increase their numbers during periods of favorable conditions whereas the great availability of resources (food plants) and apparent escape from natural enemies in their native home range provide invasives an unhindered opportunity to colonize new habitats.

The vast array of arthropods collected in the field is a good indication of the richness of arthropod taxa in the corn fields not treated with insecticides in tropical conditions. This is the first report documenting arthropod community diversity and composition in stacked Bt corn hybrids in conditions that include chemical-free treatments or plots in the Philippines. In this study, the arthropods were classified into herbivores, predators, parasitoids, neutrals to determine the composition of their respective guilds. However, there is a need to assess the possible effects of stacked Bt corn hybrids on the guild as a functioning group. This will be discussed as a sequel to this paper. Moreover, the possible influence of the surrounding vegetations on arthropod diversity and abundance in the corn field and, consequently, on future IRM strategy should be further analyzed. A separate paper on this will also be presented. Results of this study also suggest that the use of Bt corn, being relatively free from chemical pesticides, tends to encourage or maintain biodiversity in corn agroecosystems. Hence, this technology may actually be more environment friendly than previously expected.

The absence of clear trend or statistically significant differences among the treatment means imply that the MON89034 (single or by itself) and MON89034xNK603 have no significant positive or negative effects on non-target arthropods in the three areas during the 2009 season. It is recommended that these results be compared to those obtained during the wet season trial.

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