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NON-TARGET ORGANISMS ON Bt CORN HYBRIDS MON89034 AND MON89034/NK603: PART 3. FUNCTIONAL GUILDS OF ARTHROPODS IN REGULATED FIELD TRIAL SITES DURING THE WET SEASON IN LUZON AND MINDANAO, PHILIPPINES[1]

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ABSTRACT

Regulated field experiments were conducted in Ilagan, Isabela and Tupi, South Cotabato to assess the effects of two new Bt corn hybrids (MON89034 expressing two insecticidal proteins, Cry1A.105 and Cry2Ab2 and MON89034/NK603 that additionally expresses glyphosate resistance) on non-target organisms (NTOs) specifically arthropods. Effects were assessed in comparison with two non-Bt corn hybrids (DK818 isogenic conventional corn and NK603 with glyphosate resistance trait) with no chemical protection. Arthropods were sampled using sweep net and vacuum, pitfall trapping, sugar- and protein-baiting and visual counting. A total of 15,970 and 31,699 individuals were collected in Isabela and South Cotabato, respectively. These arthropods are spread in 18 insect orders with 113 families for Isabela and 20 insect orders with 127 families for South Cotabato. The functional guilds (predators, parasitoids, pollinators, neutrals, non-target corn herbivores) appeared unhampered by the corn type as to their respective roles in the corn agroecosystem. The overall trend indicates that the mean number of arthropods was highest at the reproductive stage of the corn plant. The addition of NK603 in the wet season trial had no effect on the arthropod populations. In summary, the combined analysis showed that the arthropod populations were not significantly different among treatments and the levels of arthropod populations in non-Bt and Bt-corn fields did not show any significant difference. Data generated from both dry and wet season trials support the hypothesis that MON89034 and MON89034/NK603 have no effects on non-target arthropods in the corn ecosystem and suggest that there are no significant seasonal variations in the effects of Bt corn hybrids on NTOs.

Key words: abundance, Asian corn borer, Bt corn hybrids, diversity, functional guilds, MON89034, MON89034/NK603, NK603, non-target organisms, *Ostrinia furnacalis*

INTRODUCTION

The first commercially available Bt corn hybrid in the Philippines (MON810) was introduced in 2003 and provided effective control against the number one insect pest of corn, the Asian corn borer, *Ostrinia furnacalis* (Guenee). Twelve years hence, it has not shown signs of insect resistance development. As an offshoot of this success, new hybrids such as MON89034 and MON89034/NK603 with broader pest targets have been developed and introduced to corn farmers in the Philippines very recently. MON89034 is a second-generation transgenic insect-protected maize that expresses two insecticidal proteins, Cry1A.105 and Cry2Ab2, particularly protecting the corn plant from corn borer and other lepidopterous pests. On the other hand, MON89034/NK603, a stacked hybrid maize developed through traditional plant breeding, expresses the two insecticidal proteins of MON89034 and the protein providing NK603 its tolerance to the broad-spectrum herbicide glyphosate.

As required by biosafety regulations, environmental impacts of novel agricultural technologies, including the introduction and use of genetically modified organisms such as the two aforementioned products, need to be assessed prior to commercial scale production. In the agroecosystem, the biodiversity profile, which includes assessment of risks to non-target organisms (NTOs), is already a requirement in any study that aims to assess environmental impacts. In the Philippines, NTO risk assessment should be conducted for at least two seasons.

The previous trial (Lit et al., 2011 and 2012) was conducted during the dry season and the sampling methods have been evaluated in another paper (Lit et al., 2014). The dry season trial used different treatments consisting of non-Bt (DK818 Conventional with and without chemical protection) and Bt corn hybrids (MON89034 and the stacked MON89034/NK603). Results revealed that the treatments did not differ significantly in terms of the abundance, diversity and guild structures of non-target organisms. This was generally true for all the trial sites (Pangasinan, Isabela and South Cotabato), on three sampling dates (25, 60 and 85 days after planting, which coincided with the early vegetative, silking and pre-harvest stages of the corn plant, respectively) and for both foliage/canopy-dwelling arthropods as well as ground-dwelling canopy-foraging species. There were no clear statistically significant differences obtained among the treatments across sites and this suggests that the new transgenic traits have no significant positive or negative effects on the different non-target functional guilds of arthropods in the corn agroecosystems (Lit et al., 2012).

In order to assess and compare better the responses of NTOs to stacking of genes in Bt corn hybrids as against single trait hybrids, another trial was conducted to include NK603, this being one of the parental lines of the stacked hybrid corn. This second trial also completed the requirement of two-season NTO trials. This study, therefore, aimed to: (1) determine and compare the diversity of arthropods, particularly non-targets or NTOs in regulated field trial sites of MON89034 and MON89034/NK603 in Luzon and Mindanao during 2010 wet season; and (2) identify and compare the different functional guilds in the inclusive ecological communities of the abovementioned regulated field trial sites.

MATERIALS AND METHODS

Study Sites

Two study sites, one for Luzon and another for Mindanao, were approved by the Bureau Plant Industry, Department of Agriculture. For Luzon, the experimental site was located inside the Cagayan Valley Integrated Agricultural Research Center (CVIARC) Experiment Station, Department of Agriculture Region 2, Barangay San Felipe, Ilagan, Isabela. It has an elevation of 45 m asl and coordinates 17°07'51.1" North and 121°53'15.2" East. The surrounding farms, about few meters away, were mostly planted to corn at varying stages of maturity. For Mindanao, the site was in Lorenzo Farm, Barangay Crossing Rubber, Tupi, South Cotabato, with an elevation of 232 m asl and coordinates 6°20'55.4" North and 124°55'21.6" East. It was inside a vast track of farmland planted to annual and perennial crops and within the view of Mt. Matutum. This area was less affected by the El Niño phenomenon in Mindanao. Compared to the previous NTO trials, the four corners of the confined field were cleared, except for the coconut grove. These lots were grown to vegetable crops regularly sprayed with chemical pesticides.

Experimental Plots and Design

The field trials for each location consisted of four (4) treatments: T1 - Non-Bt NK603 (Corn Event NK603; Trade Name - Roundup Ready™ Corn with herbicide (glyphosate) tolerance trait); T2 - Non-Bt conventional hybrid corn DK818; T3 - Transgenic hybrid Bt corn MON89034; and T4 - Stacked Bt corn MON89034/NK603. Planting dates for the two sites were scheduled to synchronize/harmonize monitoring activities in the two localities.

The hybrids were planted in the confined trial following a distance of 0.75-0.80 m between rows and 0.20 cm between hills. The plots were laid out in a Randomized Complete Block Design (RCBD) with three replications. Each plot had 10 rows x 10 m which included two border rows plus two pollinator rows of the conventional hybrids. The surrounding area of the confined trial was planted to 5-m rows of non-Bt conventional hybrid field corn to minimize potential edge effects. All the necessary cultural management practices in corn growing were employed, except that no insecticide was applied throughout the duration of the trials.

In conformity with established protocol on biosafety procedures for Bt corn planting, the plants in experimental plots at Ilagan, Isabela were detasseled at 55 days after planting. This procedure was strictly followed to enforce isolation and prevent pollen dispersion from the transgenic Bt corn plants to the neighboring farmer's cornfields surrounding the experimental area. To provide pollen for the experimental plots, two rows of the non-Bt conventional hybrid corn served as the buffer plants for the trial as mentioned above. The study was conducted from May to August 2010.

Collection of Arthropods

Collected individuals were classified into: a) foliage/canopy-dwelling and b) ground-dwelling canopy-foraging arthropods. Foliage/canopy dwellers consisted mainly of arthropods collected by sweep net and vacuum sampling, as described by Lit et al. (2014a), and/or recorded by visual counting. The latter was supplemented with collection of specimens and/or photodocumentation particularly for species that are unfamiliar to the observer. On the other hand, ground-dwelling canopy-foragers consisted of arthropods collected through pitfall traps and sugar- and protein-baiting (largely for ants).

Arthropods were collected/observed from the eight inner rows of each 10-row plot using the different methods. Sampling was done at 25-30 (early vegetative stage), 55-60 (reproductive, pre-tassel/silking stage) and 80-85 (pre-harvest, mature stage) days after planting (DAP). The insect collection methodologies, sorting, preservation and identification of collected specimens, data management and analyses, digital photodocumentation, voucher specimens deposition and storage followed the same procedures as those in Lit et al. (2011) and evaluated for efficiency or effectiveness by Lit et al. (2014b).

RESULTS AND DISCUSSION

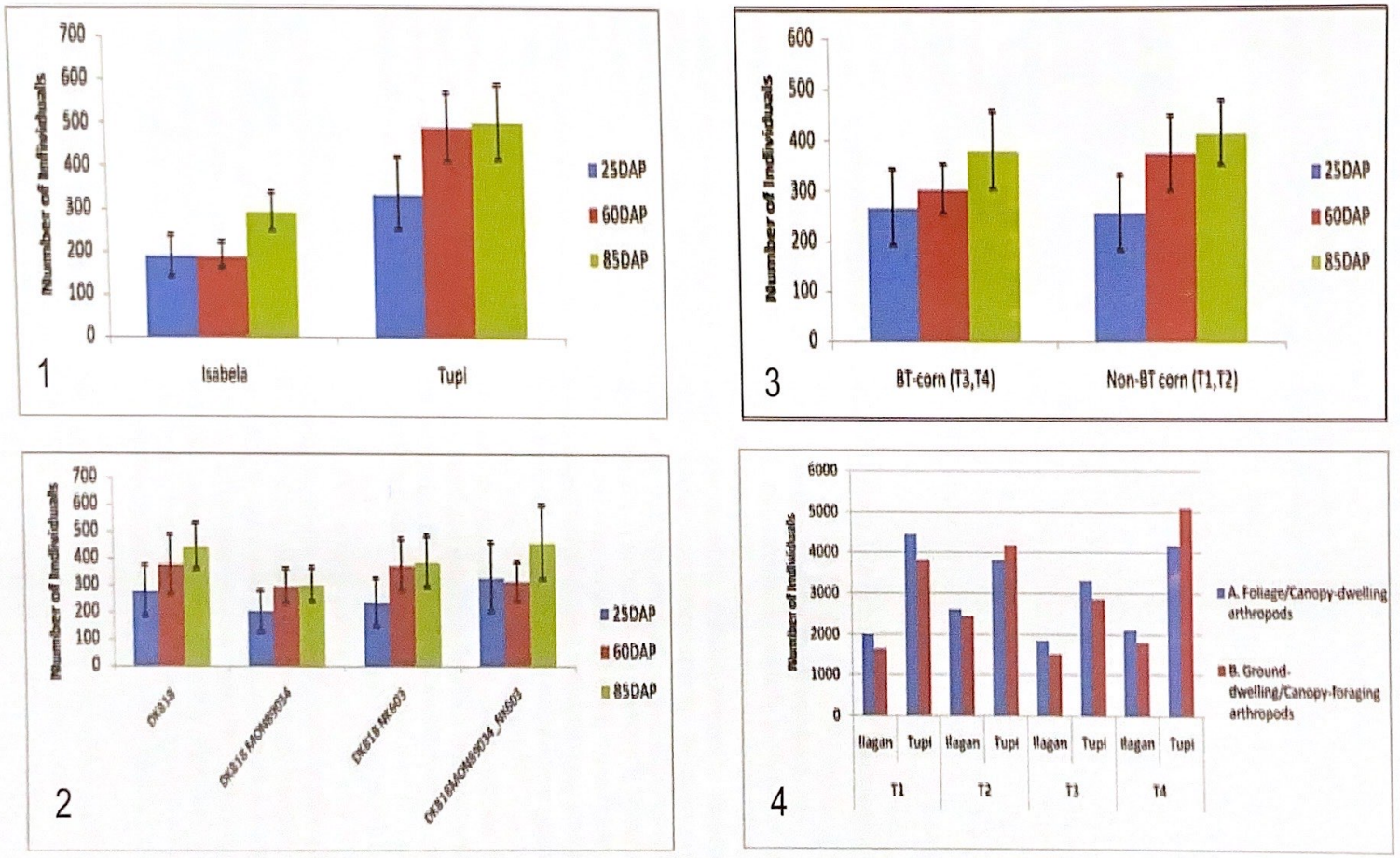
General Trends in Arthropod Composition

Plant phenology and site. In general, arthropod populations increased as the corn plants matured (or across sampling periods indicated as DAP), in both sites in Ilagan, Isabela and Tupi, South Cotabato (Figure 1). There were more arthropods in the corn field in Tupi, South Cotabato than in Ilagan, Isabela with almost 500 arthropods collected at 60 and 85 DAP. The highest Isabela populations at 85 DAP corresponds to the lowest South Cotabato populations at 20 DAP.

Treatment. Arthropod populations in all treatments increased across sampling periods except for the stacked corn (MON89034/NK603), which had more arthropods at the vegetative stage (Figure 2). However, the differences among treatments were not significant. MON89034 had slightly fewer arthropods compared to the other corn hybrids and the arthropod population remained steady from anthesis to physiological maturity of the crop.

Corn type. Arthropod populations in Bt and non-Bt corn also increased as the plants matured and total numbers of arthropods were not significantly different between the two types of corn (Figure 3).

Microhabitat. Within a cornfield, parts of the plant above the ground (foliage or canopy) serve as a different microhabitat for a different array of target and non-target organisms compared to the ground and below-ground parts. A few, but ecologically significant groups like ants and other predators of corn pests and other insects, however, forage in the canopy although they may be ground-dwelling. The number of foliage/canopy-dwelling arthropods was higher compared to that of ground-dwelling canopy-foraging in all treatments, except for the conventional non-Bt corn and stacked



Figures 1-4. Comparative total numbers of arthropods in the two regulated corn field trial sites collected during three sampling dates/ corn plant stages, accumulated using five sampling methods: **(1.)** Comparing the two trial sites at 25, 60 and 85 days after planting (DAP); **(2.)** Arthropod abundance among the four treatments; **(3.)** Arthropod abundance on non-Bt and Bt corn; **(4.)** Total number (individuals) of foliage/canopy-dwelling and ground-dwelling canopy-foraging arthropods for the four treatments./T1-NK603; T2-Conventional; T3-MON89034; T4-MON89034/NK603

Bt corn in Tupi, South Cotabato (Figure 4). The number of ground-dwelling canopy foraging arthropods was expectedly low because of the fewer samples collected per plot. Lumping values for both sites and for all treatments, 24,388 and 23,277 individuals represent the communities of foliage/canopy-dwelling and ground-dwelling, respectively.

Arthropod Taxa on Maize

Arthropod taxa on non-Bt and Bt-corn in the NTO field trials in Isabela and South Cotabato were categorized based on ecological functional guilds. Overall, a total of 15,970 and 31,699 individuals were collected and recorded in Isabela and South Cotabato, respectively. These belong to 18 insect orders for Isabela and 20 for South Cotabato. There were 113 and 127 families for Isabela and South Cotabato, respectively. The total number of species was higher in Isabela with 231 as compared to that in South Cotabato with 212. The greatest proportion of arthropods for Ilagan, Isabela and Tupi, South Cotabato was recorded for predators, comprising 59% and 60% of the total density, respectively.

Abundance of Arthropods by Functional Guild: Foliage/Canopy-Dwellers

Predators. The abundance of foliage/canopy dwelling predators in the different treatments on all sampling dates is shown in Figure 5b. These predators include *Cheilomenes sexmaculatus* (Fabricius) (Coleoptera: Coccinellidae); *Mallada basalis* (Walker) (Neuroptera: Chrysopidae); *Micraspis* spp. (Coleoptera: Coccinellidae); *Micromus igorotus* Banks (Neuroptera: Hemerobiidae); *Proreus simulans* (Stål) (Dermaptera: Chelisochidae); *Simosyrphus scutellaris* (Fabricius) [=*Ischiodon scutellaris* (Fabricius)] (Diptera: Syrphidae). All are regarded as generalist predators although the syrphid and neuropterans are better known as predators of corn leaf aphids, planthoppers and smaller soft-bodied insects. All have also been documented as capable of preying on various stages of the Asian corn borer, especially *P. simulans*.

There was an increase in the number of predators from 25 to 60 DAP and a decrease at 85 DAP. Non-Bt NK603 had the highest mean number of predators in South Cotabato, followed by the conventional hybrid, while NK603 and MON89034 had the highest in Isabela. There were no significant differences observed among the four treatments at 85 DAP in both locations.

Parasitoids. Altogether, there was an increase in number of parasitoids at 25 to 60 DAP and a decrease at 60 to 85 DAP both in Isabela and South Cotabato. The number of parasitoids (Figure 5a) in this case appears to depend on the presence of target pests (Figure 6c) during each sampling period. The most notable among observation for parasitoids were blackened egg masses of the Asian corn borer, from which tiny *Trichogramma* parasitoids emerged a few days after they are collected and kept in large cotton-plugged test tubes in the laboratory.

Results indicate the presence of both parasitoids and pests during the three sampling periods. The abundance of parasitoids on non-Bt and Bt corn hybrids in both sites (Figure 5a) were not significantly different, except for the significantly fewer

parasitoids on DK818 conventional hybrid in Ilagan, Isabela and on the stacked hybrid at 85 DAP.

Pollinators. The abundance of foliage/canopy-dwelling pollinators like native honeybees (*Apis* sp.), stingless bees (*Tetragonula* sp.) etc. were not significantly different among all treatments, across sampling dates in both trial sites.

Neutrals. Significantly more foliage/canopy-dwelling neutrals during the whole cropping period were observed in South Cotabato (Figure 6a). The significantly fewer arthropods in Isabela may be attributed to the removal of tassels (detasseling) prior to the conduct of the second arthropod sampling at 60 DAP. The abundance of foliage/canopy-dwelling neutrals increased with plant maturity. However, there were no significant differences among the four treatments in all sampling dates for both sites.

Non-target pests/herbivores. There were significantly more foliage/canopy-dwelling non-target pests at 25 DAP (x=171) and 60 DAP (=714) in South Cotabato. Among sucking non-target pests/herbivores, the invasive corn planthopper *Stenocranus pacificus* Kirkaldy (Hemiptera: Delphacidae) and the corn leaf aphid *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) were the most commonly encountered. For chewing species, the corn silk beetle *Monolepta bifasciata* Hornstedt and the Philippine katydid *Phaneroptera furcifera* Stål (Orthoptera: Tettigoniidae) were usually observed during the silking and early vegetative stages, respectively. In both sites, the number of non-target pests were roughly similar at 85 DAP, with Isabela having numerically higher but not significantly different value. The abundance of non-target pests at each sampling period for each treatment is shown in Figure 6b. The population increased through time, with the highest readings recorded at 60 DAP in South Cotabato.

The population of non-target pests in Isabela at 60 DAP increased to only less than 100 individuals in all treatments in comparison with the mean of 714 for South Cotabato. Also during that time, the stacked hybrids registered the highest number of non-target pests but were not significantly different from those of the other corn hybrids. There was a drastic increase in the number of non-target pests in Isabela at 85 DAP, ranging from 300 to 1000 individuals, with the conventional hybrid corn having the highest number (Figure 6b).

Target pests. Significantly more target pests, i.e. various stages of the Asian corn borer *Ostrinia furnacalis* (Guenee), were observed at 60 DAP in Isabela (x=11) than in South Cotabato (=4). Target pests were few during the first reading (25 DAP) in all treatments. This increased only on non-Bt corn as the plants reached the reproductive stage and eventually declined at pre-harvest stage. The abundance values of foliage/canopy-dwelling target pests were not significantly different among treatments (Figure 6c).

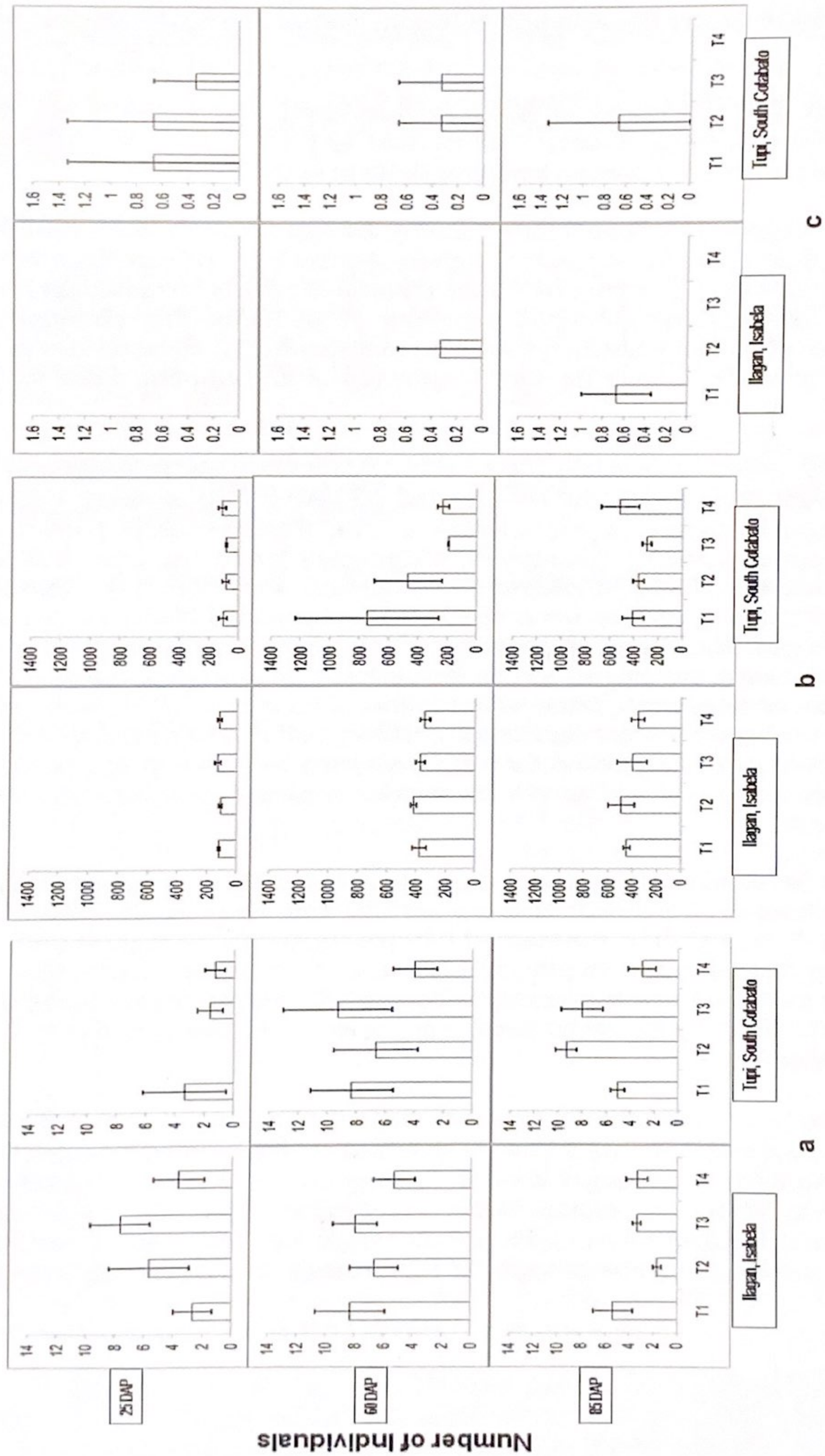


Figure 5. Comparative abundance (mean number of individuals) of foliage/ canopy-dwelling arthropods at each sampling period for each treatment in the regulated field trial sites in Ilagan, Isabela and Tupi, South Cotabato. **(a.)** parasitoids. **(b.)** predators . **(c.)** pollinators. Labels on the x-axis are treatments (corn varieties): T1 - NK603; T2 – Conventional; T3 - MON89034; T4 - MON89034/NK603.

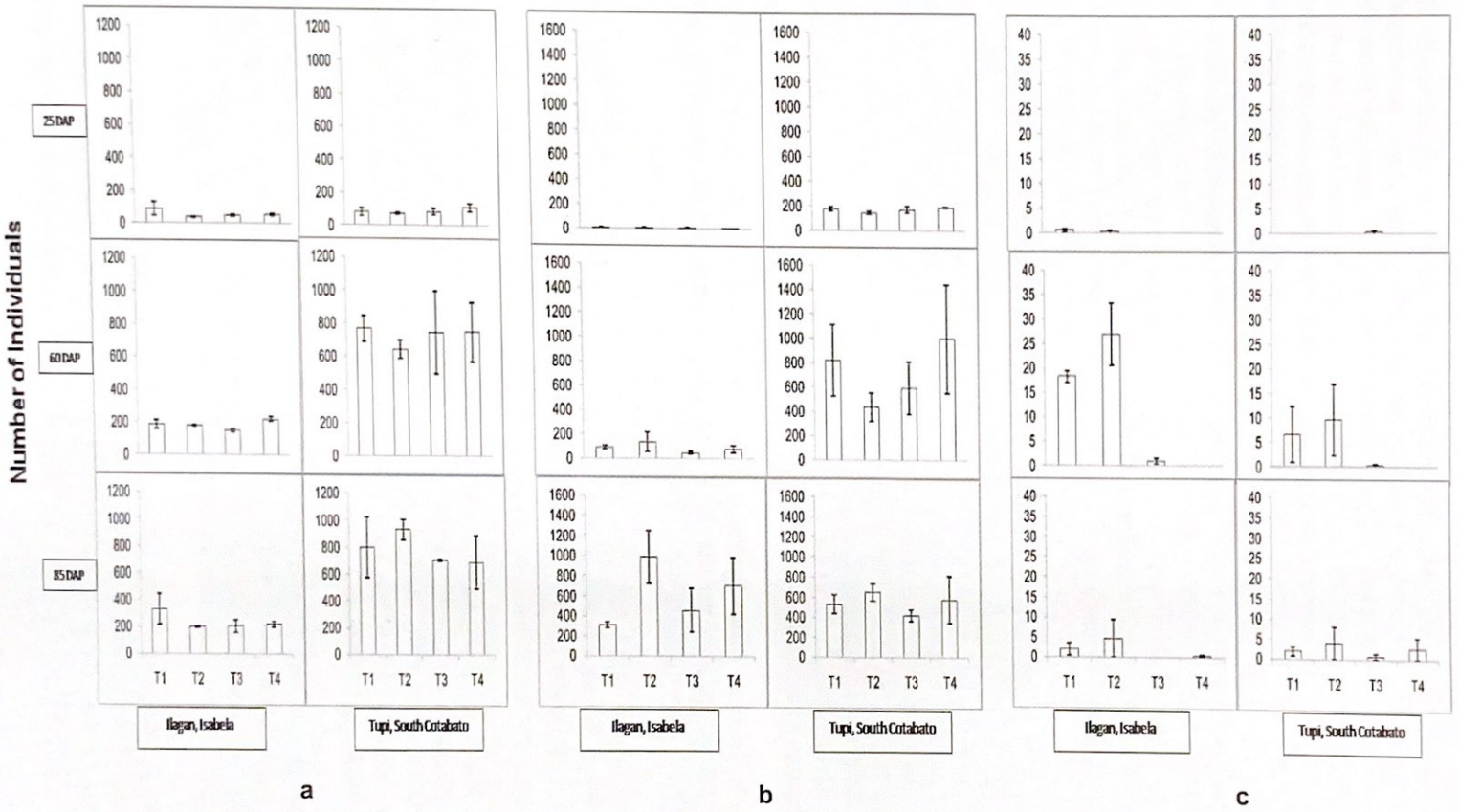


Figure 6. Comparative abundance (mean number of individuals) of foliage/canopy-dwelling arthropods at each sampling period for each treatment in the regulated field trial sites in Ilagan, Isabela and Tupi, South Cotabato. **(a.)** neutrals. **(b.)** non-target pests. **(c.)** target pests. Labels on the x-axis are treatments (corn varieties): T1 - NK603; T2 – Conventional; T3 - MON89034; T4 - MON89034/NK603.

Treatment and sampling date interaction. The number of foliage/canopy-dwelling arthropods for the four treatments and three sampling dates, averaged over two sites and three replications, can be gleaned from Figures 5-6. Results show that all the functional guilds were not significantly different among the four treatments, except for the target pests. There were significantly more target pests on non-Bt corn (conventional hybrid corn, T2 and NK603, T1) than on Bt-corn (MON89034, T3 and MON89034 x NK603, T4). In other words, abundance of NTOs did not differ significantly among all treatments and sampling dates. This suggests that Bt corn has no adverse effect on NTO populations.

Abundance of Arthropods by Functional Guild:
Ground-Dwelling Canopy-Foragers

Predators. There were significantly more ground-dwelling canopy-foraging predators in South Cotabato in all sampling periods, with number of individuals ranging from 1021 to 1612. These predators were mostly spiders, staphylinids and mirids, although predatory ants especially tropical fire ants *Solenopsis geminata* (Fabricius) were also omnipresent in all stages and plots. The predators in Isabela had dramatically declined at 60 DAP. The abundance of ground-dwelling canopy-foraging predators in the different treatments was highest on the stacked Bt-corn at 25 DAP and 85 DAP and on conventional hybrid corn at 60 DAP (Figure7a). However, there were no significant differences in abundance among treatments on all sampling dates.

Parasitoids. Isabela had significantly more parasitoids recorded in the whole season but the numbers of ground-dwelling parasitoids were not significantly different among treatments on all sampling dates (Figure 7c).

Pollinators. There were only few pollinators collected in Tupi, South Cotabato that were observed dwelling on the ground as compared to Ilagan, Isabela (Figure 7c). Nonetheless, no significant differences across sites and among treatments were observed.

Neutrals. Neutrals were significantly more numerous at 60 DAP and 85 DAP. The number of neutrals increased at 60 DAP, being most abundant on MON89034 (Figure 7b). Neutrals include arthropods not classified as non-target or target herbivores. They can be transients, vagrants and uncommon species visiting the corn field during the sampling period.

Non-target pests. Significant differences in the number of ground-dwelling canopy-foraging non-target pests were observed. There were more non-target pests in Isabela during the vegetative stage of the crop which declined as the plants matured. More non-target pests were collected in South Cotabato at 60 and 85 DAP.

Treatment x sampling date interaction. Data on number of ground-dwelling arthropods for the four treatments and three sampling dates, averaged over two sites and three replications can be gleaned from Figure 7. Only the non-target pests showed significant differences among the four treatments. There were more non-target pests on

non-Bt corn compared to Bt-corn. Ground NTO populations were not different across treatments and sampling dates which again implies that Bt corn had no effect on ground-dwelling canopy-foraging NTOs.

Combined Community of Foliage/Canopy-Dwelling and Ground-Dwelling Canopy-Foraging Corn Arthropods

Predators. The mean combined number of predators was highest at 25 DAP with South Cotabato having the highest (1710) compared to Isabela (983) (Figure 9a). Consistently, there were significantly more predators in South Cotabato in the two succeeding samplings (Figure 8a). The diversity of surrounding vegetation in South Cotabato may have had a significant effect on the abundance of arthropods in the area. The confined field trial was surrounded by other farm lots planted to different crops and other naturally-growing vegetation. The whole area of Tupi, South Cotabato can be described as an agroforestry area where fruit trees and forestry species served as the buffer plants in between small and big farms. In the middle of these farms are plots of vegetables or young fruit trees. The climate is distinct during the onset of the wet season where it is sunny in the morning and rainy in the afternoon. This condition might have contributed to the generally higher number of arthropods as compared to Isabela which is an experimental station. Due to a previous occurrence of El Niño phenomenon, the surrounding fields were bare before planting and only weeds were growing at 25 DAP. The fields were later planted to corn and then other weeds became abundant in the area. The common predators for both trial sites were spiders, mirid bugs, carabid beetles, earwigs and lygaeids.

Parasitoids. The mean combined number of parasitoids was significant only at 25 DAP, there being more in Isabela than in South Cotabato (Figure 8a). There were no significant differences in the number of parasitoids at 60 and 85 DAP between the two sites but the number of parasitoids declined through time. There were no significant treatment differences in the number of parasitoids at 25 and 60 DAP (Figure 9b). However, a significantly lower mean number of parasitoids was observed at 85 DAP on conventional hybrids in Isabela and on stacked Bt corn in South Cotabato. The fewer parasitoids in Isabela on conventional corn may be explained by the relatively fewer potential hosts among the target and non-target pests. On the other hand, the least number of parasitoids on stacked Bt corn maybe be attributed to the absence of pest species therein during the last two sampling periods.

Pollinators. There were no observed site differences in the mean number of pollinators for all sampling dates and treatments. The number of pollinators was relatively low all throughout the sampling periods (Figure 9c). Adult syrphids or hover flies, which are known as pollinators, were classified under predators together with their larvae and pupae as the immature stages are effective predators of aphids and other soft-bodied herbivores.

Neutrals. The combined number of neutrals increased over time and peaked at 85 DAP in South Cotabato (Figure 10a). The mean numbers at 25 DAP ranged from 50 to 150 individuals and no significant difference was observed between the two sites. Those at 60 DAP in the two sites were highest and ranged from 200 to 780 individuals

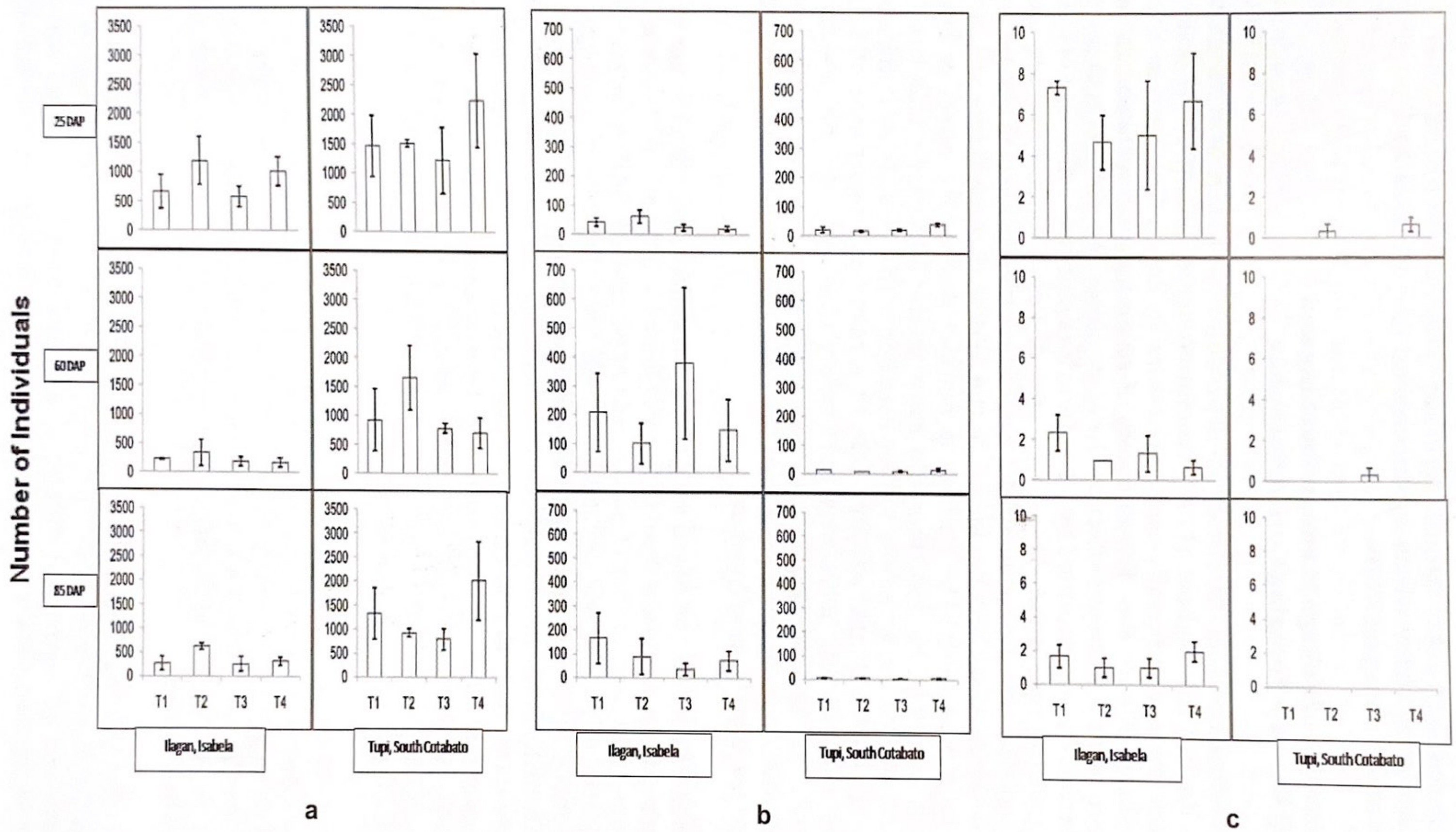


Figure 7. Comparative abundance (mean number of individuals) of ground-dwelling canopy-foraging arthropods at each sampling period for each treatment in the regulated field trial sites in Ilagan, Isabela and Tupi, South Cotabato. **(a.)** predators. **(b.)** neutrals. **(c.)** pparasitoids. Labels on the x-axis are treatments (corn varieties): T1 - NK603; T2 – Conventional; T3 - MON89034; T4 - MON89034/NK603.

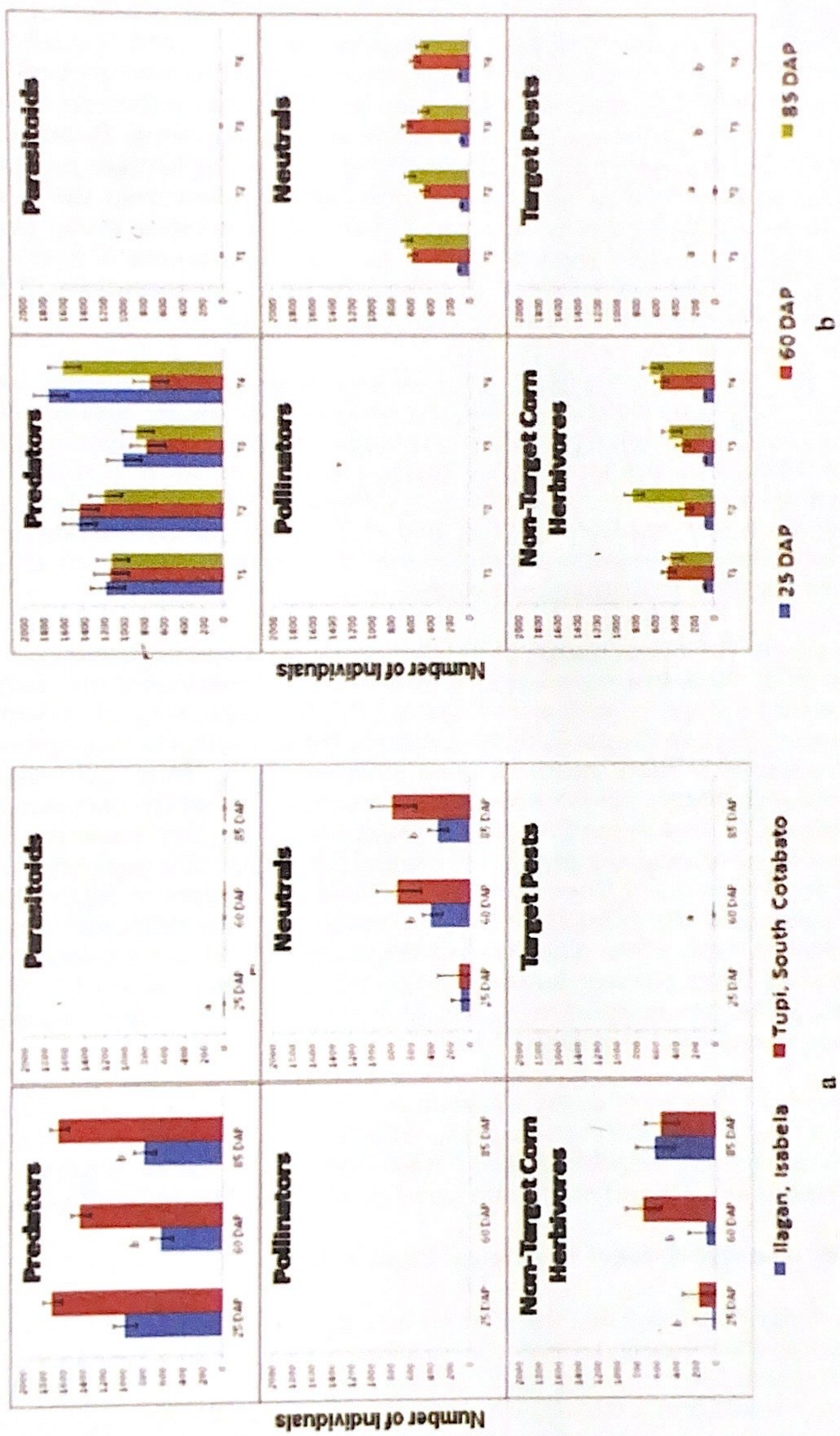


Figure 8. Mean number of combined foliage / canopy-dwelling and ground-dwelling arthropds: **(a.)** observed at two sites and three sampling peiods, average of four treatments and three replications per site. **(b.)** observed at the four treatments, averaged over two sites and three replications per site: T1-NK603; T2-Conventional; T3-MON89034; T4-MON89034/NK603.

with South Cotabato having higher number of neutrals. Their abundance steadily increased probably during the grain-filling stage of the corn plant toward the third sampling period. The presence of more foliage/canopy-dwelling and ground-dwelling canopy-foraging neutrals in South Cotabato may be attributed to the role of tassels in the abundance of arthropods in maize during the second sampling. Detasseling was not performed in the confined trials in South Cotabato and arthropod sampling was done after anthesis when pollen had already been shed. Pollen from the tassel may serve as food for many arthropods in the corn field and this food source may even invite other arthropods from outside the confined trial. The populations of neutrals were mostly dipterans with the Chloropidae (frit flies) being most abundant. Other flies included species of Phoridae and Chironomidae among others.

Non-target pests. The number of non-target pests significantly increased starting at 25 to 60 DAP in South Cotabato as indicated by higher means (173 at 25 DAP; 740 at 60 DAP). The number of non-target pests increased in Isabela only at the later stage (85 DAP) when the trend was already declining in South Cotabato (Figure 10b). The same explanation i.e. the effect of detasseling done in Isabela on corn arthropods as above, is ascribed to the higher number of arthropods in South Cotabato. Some of the non-target pests include the corn semi-looper, corn armyworm, corn planthopper, aphids and other herbivores.

Target pests. The mean numbers of target pests were not significant at both 25 and 85 DAP in both sites, but significant at 60 DAP. There were only few target pests during the vegetative stage in both sites (Figure 10c), but more were observed at post tasseling in Isabela than in South Cotabato. Among the hybrids, the conventional and NK603 had consistently more pests in both sites especially from anthesis to pre-harvest. The identified target pests were the Asian corn borer and the corn earworm. At earlier sampling dates, leaf (whorl) feeding damage mostly by corn earworm observed only on the two conventional corn hybrids, ranged from 1 to 22% (x=6.68) for NK603 and from 1.92 to 14% (x=6.19). There were no leaf feeding damages on MON89034. The above results confirmed the data taken on the resistance of the different corn hybrids taken at 85 DAP in both sites. The two conventional hybrid corn treatments were susceptible to corn borer damage as reflected in the resistance values for T1 and T2 (Table 1). This is further supported by the higher number of broken tassels where NK603 had more compared to the DK818 conventional corn hybrid.

Treatment x sampling dates interaction. Overall, the interactions between treatments and sampling dates among the different functional guilds showed no significant difference except for target pests at 60 DAP (Figure 8a). Non-Bt corn (T1 and T2) had significantly more target pests compared to Bt-corn (T3 and T4) (Figure 8b).

Comparing Dry and Wet Season Regulated Field Trials

Based on the above results, the NTO populations were not significantly different in all treatments and sampling dates across trial sites during the wet season. Analysis of arthropod abundance using the six functional guilds in two habitats (foliage/canopy-dwelling arthropods and ground-dwelling, canopy-foraging arthropods) including the combination of the two dwellings revealed varying responses.

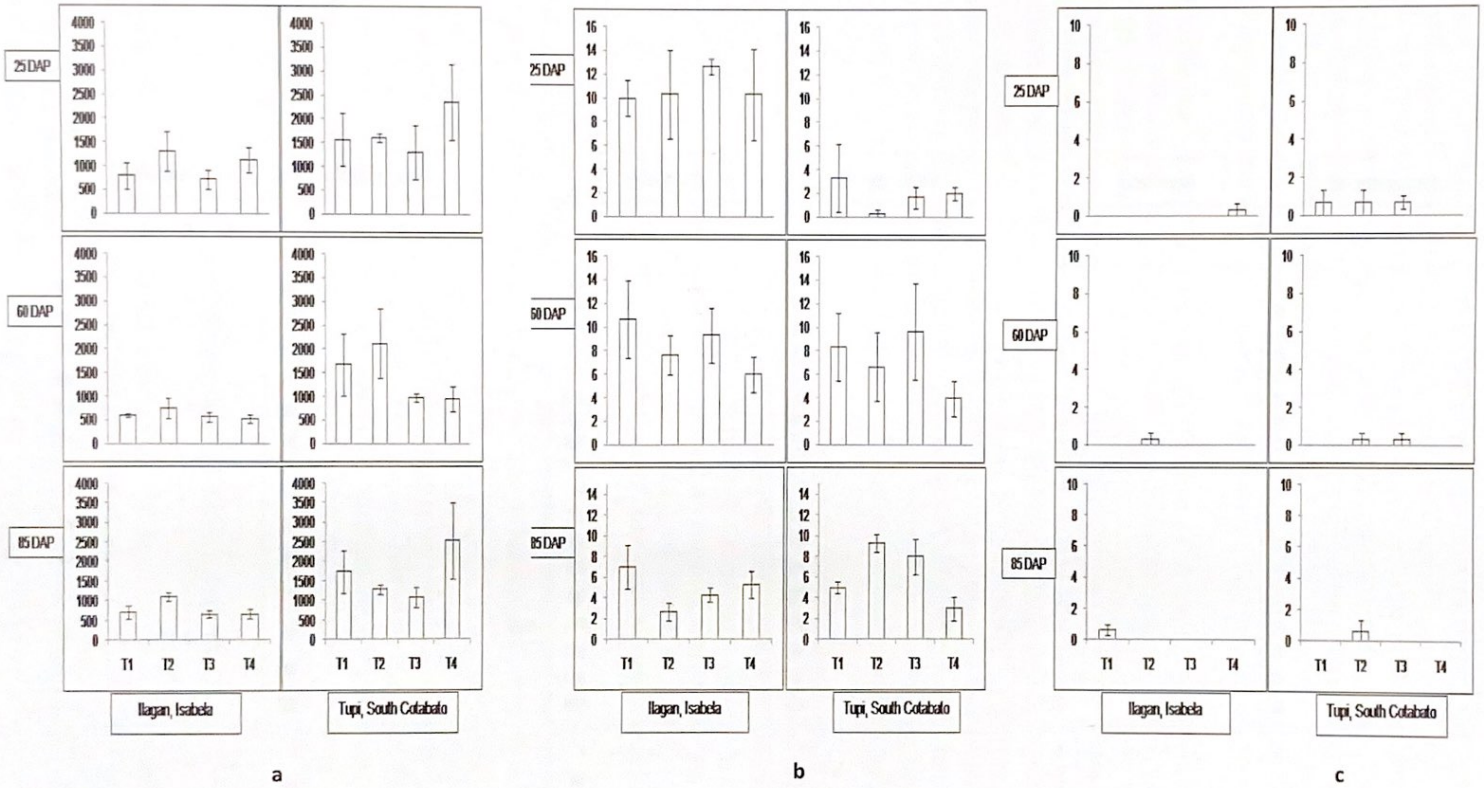


Figure 9. Comparative abundance (mean number of individuals) of combined foliage/canopy-dwelling and ground-dwelling canopy-foraging arthropods, namely: **(a.)** predators **(b.)** parasitoids and **(c.)** pollinators, at each sampling period for each treatment in the regulated field trial sites in Ilagan, Isabela and Tupi, South Cotabato. Labels on the x-axis are treatments (corn varieties): T1– NK603, T2- Conventional, T3- MON89034 and T4- MON89034/NK603.

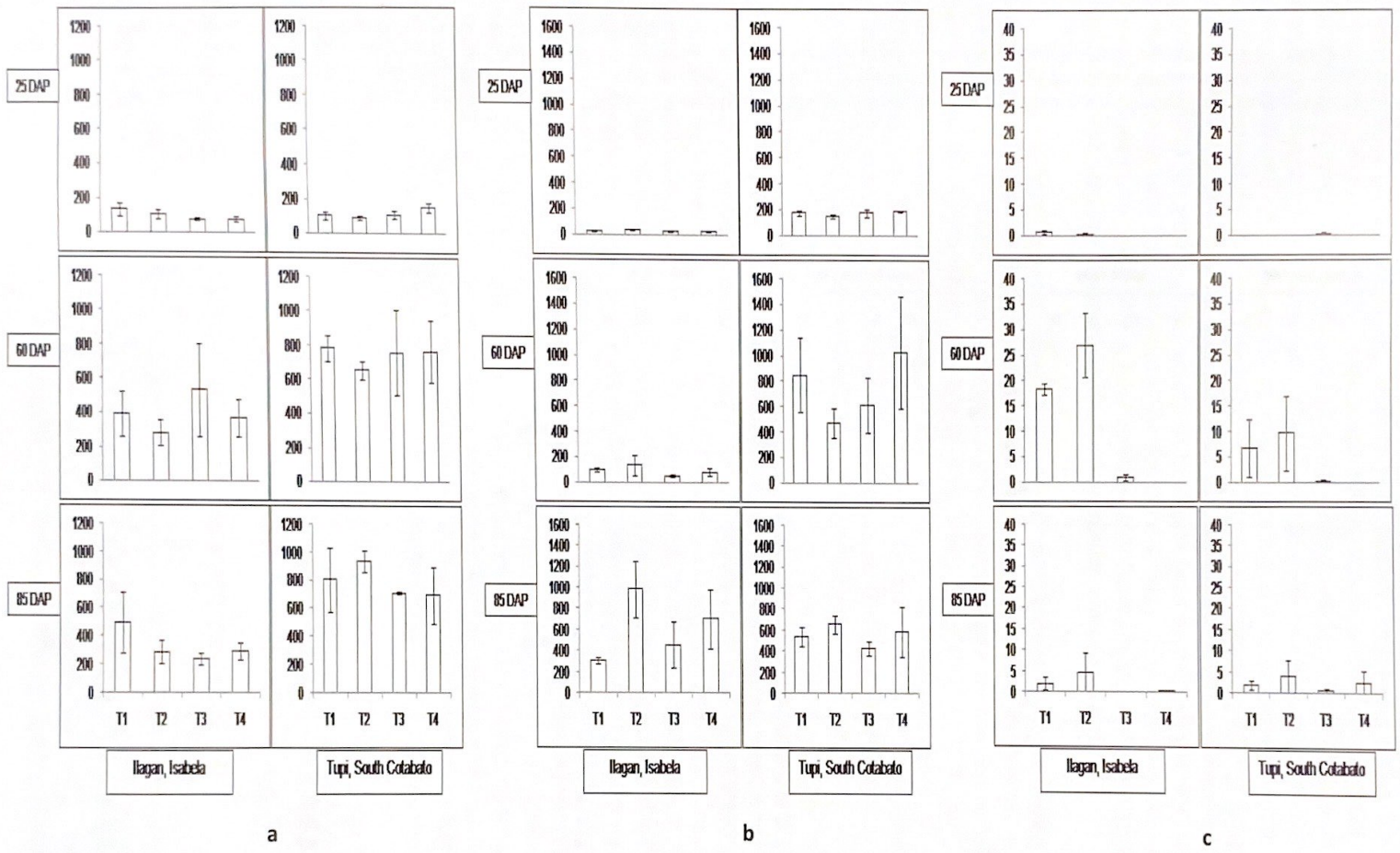


Figure 10. Comparative abundance (mean number of individuals) of combined foliage/canopy-dwelling and ground-dwelling canopy-foraging arthropods, namely: **(a.)** neutrals **(b.)** non-target pests and **(c.)** target pests, at each sampling period for each treatment in the regulated field trial sites in Ilagan, Isabela and Tupi, South Cotabato. Labels on the x-axis are treatments (corn varieties): T1–NK603, T2- Conventional, T3- MON89034 and T4- MON89034/NK603.

The number of individuals in the different functional guilds in the foliage/canopy-dwelling arthropods did not show consistent significant differences among sites, treatments and their interactions. The same results were observed for ground-dwelling canopy-foraging arthropods and no consistent trends were also observed when the foliage/canopy-dwelling and ground-dwelling canopy-foraging arthropods were combined. To summarize the individual results of the above, no consistent significant differences in the population of foliage/canopy-dwelling and ground-dwelling canopy-foraging arthropods were found among treatments and between corn types.

Results also revealed that using this classification, there was also no consistent trend in the effects of MON89034 and the stacked Bt corn hybrids on arthropod composition. Numerous reports showed that functional guilds were not affected by Bt corn (Daly and Buntin 2005, Dively 2005, Reyes 2005, OECD 2007, Fariños et al. 2008). The extensive data generated in this part of the study only showed that there is a need to examine more closely the role of functional guilds in corn ecosystems especially the role of predators, neutrals and non-target herbivores/pests. It will also demonstrate the potential of Bt crops to increase the population of natural enemies and contribute significantly in biological control of arthropod pests. The functional guilds (predator, parasitoids, pollinators, neutrals, non-target pests) appeared unhampered by the new corn types as to their respective roles in the corn agroecosystem.

These results are similar to those obtained for the dry season (Lit et al., 2011 & 2012) (see Figures 11-14). This indicates that Bt-corn had no adverse effect on the occurrence and abundance of the non-target foliage/canopy dwelling and ground-dwelling canopy-foraging arthropods. Whereas there are differences in the species composition among the various guilds as well as among the arthropod communities in corn fields in Luzon and Mindanao, the obtained trends in the two trials indicate continued abundance and functioning of naturally-occurring biological control agents as well as pollinators and other arthropods.

The addition of NK603 in the wet season trial had no effect on the overall arthropod populations. The combined analysis of foliage/canopy-dwelling and ground-dwelling canopy-foraging arthropods showed that the arthropod populations were not significantly different among treatments and the levels of arthropods between non-Bt and Bt-corn did not show any significant difference, except for the expected occurrence of lepidopterous pests particularly the Asian corn borer on non-Bt corn hybrids. Data generated from both dry and wet season trials support the hypothesis that MON89034 and MON89034/NK603 have no effects on non-target arthropods in the corn ecosystem and suggest that there are no significant seasonal variations in the effects of Bt corn hybrids on NTOs.

SUMMARY AND CONCLUSION

This study assessed the effects of Bt corn, as single event and as stacked upon herbicide-resistant hybrid, on non-target arthropods. Utilizing various methods of sampling, significant results were generated in a manner and effort that was as comprehensive as possible. A total of 15,970 and 31,699 individuals were collected in Isabela and South Cotabato, respectively. These arthropod taxa were spread in 18

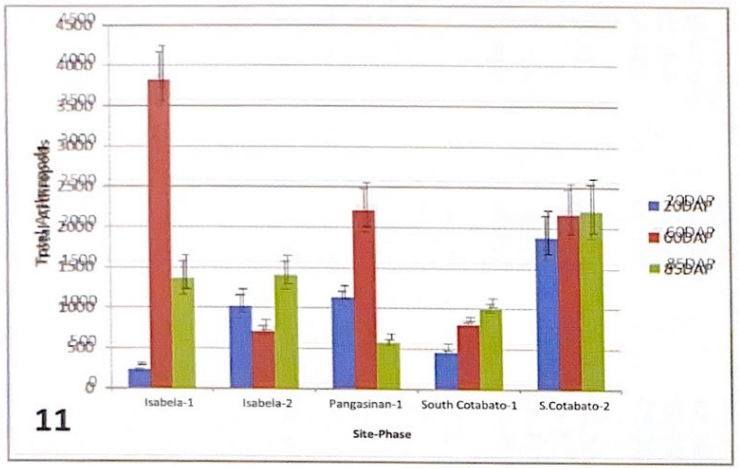
insect orders with 113 families for Isabela, and 20 insect orders with 127 families for South Cotabato. Based on ecological functional guilds, the greatest proportion of arthropods in Isabela was recorded among predators comprising 61%. For South Cotabato, predators, neutrals and non-target pests occurred in almost equal thirds. The vast array of arthropods collected in the field is a great indication of the richness of arthropod taxa in the corn fields under tropical conditions like the Philippines. Their continued existence suggests unhampered reproduction and performance of ecological functions.

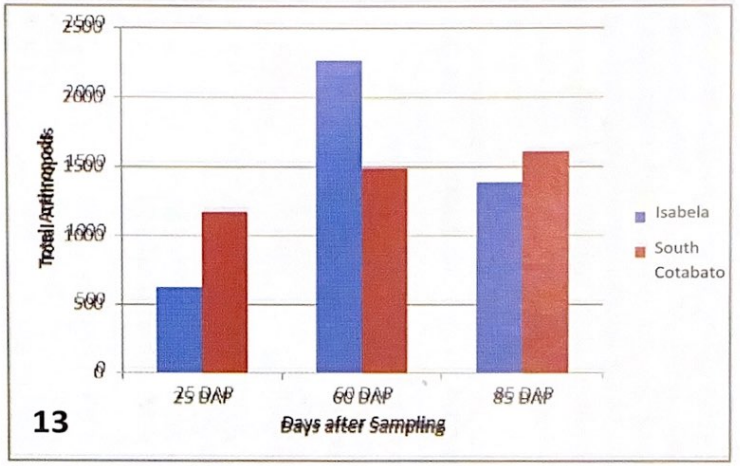
The general trends in the composition of arthropods in non-Bt and Bt corn fields showed that the population significantly increased with phenological age, probably because the increase in biomass consequential to plant growth provided greater amount of resources in terms of food and microhabitat to the arthropods. More arthropods were collected in Tupi, South Cotabato but the differences among treatments and between corn types (non-Bt and Bt corn) in both sites were not significant. As in the previous study during the dry season, by integrating the results on abundance and diversity with the functional guilds, it was concluded that based on available evidences from the wet season, the stacked Bt corn hybrids do not affect the NTOs. The results from the wet season in this study and those from the dry season (Lit et al., 2011 & 2012) were similar, suggesting that there have been no distinct seasonal variations or differences in the effects of the treatments on NTOs. Species compositions may differ but the general trends in abundance and diversity of non-target arthropods are similar. In general, the results may tend to support that biodiversity is enhanced with Bt corn whether as single or as stacked trait.

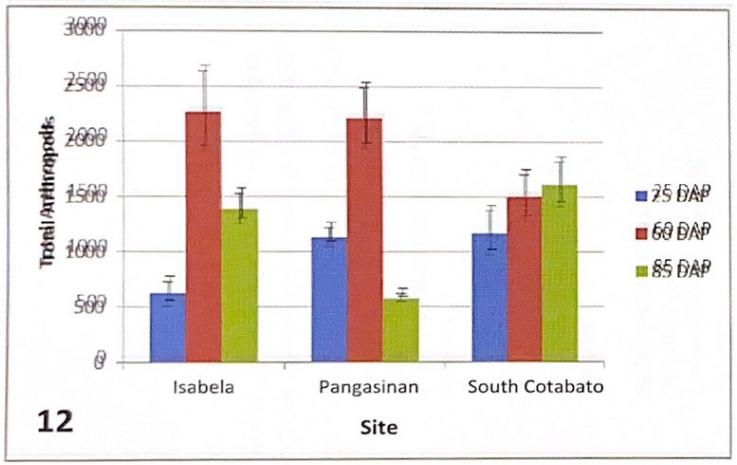
Table 1. Mean combined number of foliage/canopy-dwelling and ground-dwelling canopy-foraging arthropods in regulated field trials for wet season assessment of effects of new Bt corn hybrids on non-target organisms involving four treatments taken on three sampling dates (days after planting, DAP), averaged over two sites and three replications per treatment/site.

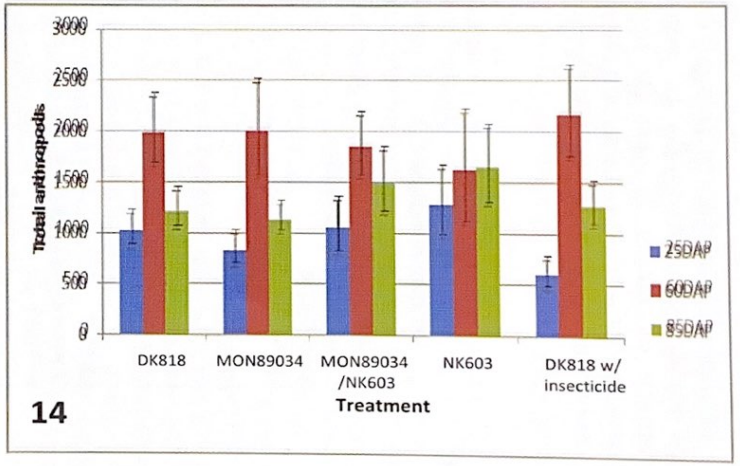
Functional Guilds	Treatments (corn varieties)	25 DAP	60 DAP	85 DAP
Predators	NK603	1178.33	1140.00	1121.00
	Conventional (DK818)	1456.00	1440.67	1191.67
	MON89034	1011.00	772.33	867.17
	MON89034/NK603	1743.50	736.17	1599.17
Parasitoids	NK603	6.67	9.50	6.00
	Conventional (DK818)	5.33	7.17	6.00
	MON89034	7.17	9.50	6.17
	MON89034/NK603	6.17	5.00	4.17
Pollinators	NK603	0.33	0.00	0.33
	Conventional (DK818)	0.33	0.33	0.33
	MON89034	0.33	0.17	0.00
	MON89034/NK603	0.17	0.00	0.00
Neutrals	NK603	117.83	588.83	649.83
	Conventional (DK818)	96.83	468.00	613.00
	MON89034	91.67	644.33	476.33
	MON89034/NK603	113.33	567.33	494.83
Non-Target Pests	NK603	105.67	473.83	428.17
	Conventional (DK818)	93.33	303.17	827.17
	MON89034	103.00	331.67	446.00
	MON89034/NK603	109.33	554.50	646.33
Target Pests	NK603	0.33	12.50 a	2.00
	Conventional (DK818)	0.17	18.33 a	4.33
	MON89034	0.17	0.67 b	0.33
	MON89034/NK603	0.00	0.00 b	1.50

Unmarked means or those followed by the same letter are not significantly different at 5% level by adjusted Tukey's test on LS means.









Figures 11-14. Combined analyses of dry and wet season regulated field trials in Luzon and Mindanao to assess effects of new Bt corn hybrids MON89034 and MON89034/NK603 on non-target arthropods. **(11.)** Total canopy-dwelling and ground-dwelling canopy-foraging arthropod populations, average of four treatments. **(12.)** Total arthropods (Mean counts: Isabela and Tupi N = 24 for two trials; Pangasinan N= 12 for one trial only). **(13.)** Total number of arthropods (Mean of two trials) in the different sampling periods in Ilagan, Isabela and Tupi, South Cotabato (F value = 13.08; p=0.001). **(14.)** Total canopy-dwelling and ground-dwelling canopy-foraging arthropod populations, averaged over sites and two trials.

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