

## Effects of *Bacillus thuringiensis* on non-target herbivore and natural enemy assemblages in tropical irrigated rice

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Endotoxins from *Bacillus thuringiensis* (*Bt*) produced in transgenic pest-resistant *Bt* crops are generally not toxic to predatory and parasitic arthropods. However, elimination of *Bt*-susceptible prey and hosts in *Bt* crops could reduce predator and parasitoid abundance and thereby disrupt biological control of other herbivorous pests. Here we report results of a field study evaluating the effects of *Bt* sprays on non-target terrestrial herbivore and natural enemy assemblages from three rice (*Oryza sativa* L.) fields on Luzon Island, Philippines. Because of restrictions on field-testing of transgenic rice, *Bt* sprays were used to remove foliage-feeding lepidopteran larvae that would be targeted by *Bt* rice. Data from a 546-taxa Philippines-wide food web, matched abundance plots, species accumulation curves, time-series analysis, and ecostatistical tests for species richness and ranked abundance were used to compare different subsets of non-target herbivores, predators, and parasitoids in *Bt* sprayed and water-sprayed (control) plots. For whole communities of terrestrial predators and parasitoids, *Bt* sprays altered parasitoid richness in 3 of 3 sites and predator richness in 1 of 3 sites, as measured by rarefaction (in half of these cases, richness was greater in *Bt* plots), while Spearman tests on ranked abundances showed that correlations, although significantly positive between all treatment pairs, were stronger for predators than for parasitoids, suggesting that parasitoid complexes may have been more sensitive than predators to the effects of *Bt* sprays. Species accumulation curves and time-series analyses of population trends revealed no evidence that *Bt* sprays altered the overall buildup of predator or parasitoid communities or population trajectories of non-target herbivores (planthoppers and leafhoppers) nor was evidence found for bottom-up effects in total abundances of non-target species identified in the food web from the addition of spores in the *Bt* spray formulation. When the same methods were applied to natural enemies (predators and parasitoids) of foliage-feeding lepidopteran and non-lepidopteran (homopteran, hemipteran and dipteran) herbivores, significant differences between treatments were detected in 7 of 12 cases. However, no treatment differences were found in mean abundances of these natural enemies, either in time-series plots or in total (seasonal) abundance. Analysis of guild-level trajectories revealed population behavior and treatment differences that could not be predicted in whole-community studies of predators and parasitoids. A more conclusive test of the impact of *Bt* rice will require field experiments with transgenic plants, conducted in a range of Asian environments, and over multiple cropping seasons.

**Keywords:** *Bacillus thuringiensis* / food web / *Oryza sativa* / tropical irrigated rice / arthropod community dynamics / ecological statistics / biological control / non-target impacts

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## INTRODUCTION

Genetic engineering of crops with *Bt* toxin genes has made it possible to develop cultivars with high levels of resistance to pests in the insect orders Lepidoptera and Coleoptera (Shelton et al., 2002). The introduction of this new technology raises several questions concerning impacts on agroecosystems, including direct toxicity to non-target organisms and more complex effects on the invertebrate community resulting from the removal of target herbivores (Letourneau and Burrows, 2002). Results from most laboratory and field studies suggest that *Bt* crops and *Bt* sprays are generally not toxic to beneficial arthropods and other non-target organisms (Dale et al., 2002; but see Hilbeck, 2002). However, the general aim of these studies has been to measure impacts of *Bt* crops on selected insect pests and pollinators, and their principal natural enemies. Limited information is available to show whether *Bt* technology affects entire communities of organisms.

Investigating the effects of *Bt* crops on the community ecology of an agroecosystem, including aspects such as biological control of non-target pests, requires large-scale field experiments to buffer against edge effects, to maintain patch sizes large enough for wide-ranging species, and to evaluate whether manipulations have impacts on neighboring sites (Carpenter, 1990; Likens, 1985; Schindler, 1987). Such large-scale, community-level studies have been initiated in crops where *Bt* cultivars have already been released over a wide region, such as cotton (*Gossypium hirsutum* L., Fitt and Wilson, 2002). However, similar field experiments cannot be conducted for crops that have not yet been approved for field testing or for which field tests are limited to small plots. This is the case for *Bt* rice (*Oryza sativa* L.) in most of Asia, where only China (Tu et al., 2000; Ye et al., 2001) and India (S.K. Raina, personal communication) have conducted small-scale field-testing. Field testing of *Bt* rice in Asia will probably remain limited for several more years because of biosafety regulations and the hesitancy of governments to endorse wide-scale planting of transgenic crops before their ecological effects are better understood.

The principal target pests for control by *Bt* rice in Asia are lepidopterous insects such as the yellow stem borer *Scirpophaga incertulas* (Walker) and striped stem borer *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae). Due to their wide distribution and chronic occurrence, stem borers are one of the most important insect pests of rice in terms of yield loss (De Datta, 1981; Reissig et al., 1986; Savary et al., 2000; Way et al., 1991). Numerous

foliage-feeding lepidopterans also occur in rice fields in Asia. Among the most abundant species are the leaffolders *Marasmia patnalis* Bradley and *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae). Leaffolders cause highly visible damage but rarely cause yield loss because rice can recover from extensive loss of foliage (Heong et al., 1998). Laboratory tests show that all four of these stem borer and leaf folder species are sensitive to Cry1Ab (Aguda et al., 2001; Alinia et al., 2000), the *Bt* toxin of greatest interest for transgenic deployment.

A potential problem in deploying *Bt* technology on a large scale in rice is that elimination of the targeted lepidopteran pests may deplete generalist natural enemies that effectively control secondary pests, thus unleashing them from biological control and inducing secondary pest outbreaks. The critical role of biological control in suppressing secondary pests in tropical rice was demonstrated by the devastating outbreaks of the rice brown planthopper *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) that occurred following the introduction of insecticides in the 1970s (Gallagher et al., 1994). Natural enemies of *N. lugens* alone, originally estimated at 66 taxa from four Philippine provinces (IRRI, 1979), presently include 169 taxa from irrigated and rainfed sites from throughout the Philippines (K.G.S., unpublished data). The diversity and ecology of ricefield invertebrates in the Philippines have been well documented (e.g. Cohen et al., 1994; Heong et al., 1991; 1992; Schoenly et al., 1996a; 1996b; 1998; Settele et al., 1993; Simpson et al., 1993a; 1993b; 1994a; 1994b). A food web of irrigated and rainfed rice (at and above the water line) has been developed, whose present cumulative version contains 546 rice-associated taxa and over 9 300 trophic linkages, gathered from 23 sites in the Philippines (Cohen et al., 1994; Schoenly et al., 1996a). The unique body of knowledge that has been assembled for the rice-field invertebrate community in the Philippines enables the impact of *Bt* rice to be assessed with a scope and sensitivity that is not possible in any other part of Asia. Recognizing that several years may lapse before *Bt* rice is deployed in the Philippines, we initiated a study to evaluate the ecological effects of the *Bt* technology. Because the Philippine government currently prohibits the planting of *Bt* rice, we used *Bt* sprays in lieu of transgenic plants. *Bt* sprays and *Bt* cultivars act similarly in eliminating foliage-feeding lepidopteran larvae, but *Bt* sprays, unlike *Bt* plants, do not control lepidopterous stem borers. *Bt* sprays result in the deposition of materials not present in *Bt* plants, such as *Bt* spores and inert

formulation ingredients; as such they may offer an additional food source (to invertebrates) that may increase secondary productivity. In theory, however, both *Bt* sprays and *Bt* plants could disrupt biological control of non-target pests such as the brown planthopper by forcing emigration or reduced population growth of generalist predators or hyperparasitoids affected by elimination of their lepidopteran prey and hosts.

This study is the first to combine food-web and ecological approaches to evaluate the effects of *Bt* crops on pest-enemy interactions at the guild and community levels. Two steps were employed to investigate assemblage-wide impacts of *Bt* sprays on non-target herbivore and natural enemy abundances. First, we investigated broad (community-wide) patterns in predator and parasitoid abundance using rank-abundance curves, time-series plots, and conventional and ecological statistics. The second step involved the same methods to investigate effects of *Bt* sprays after natural enemies were sorted into two guilds, namely, natural enemies that take only foliage-feeding lepidopterans in their diet or host range (hereafter called “natural enemies of foliage-feeding lepidopterans”), and natural enemies that take non-lepidopteran herbivores (*i.e.*, homopterans, hemipterans and dipterans) and other organisms (hereafter called “natural enemies of non-lepidopteran herbivores”). The four aims of this research were to document if *Bt* sprays: (i) impact overall natural enemy abundances (predators and parasitoids); (ii) differentially impact natural enemies of foliage-feeding lepidopteran and non-lepidopteran herbivores; (iii) affect non-target herbivores; and (iv) reshape trophic structure by inducing bottom-up effects.

## RESULTS

### Validation of the experiment

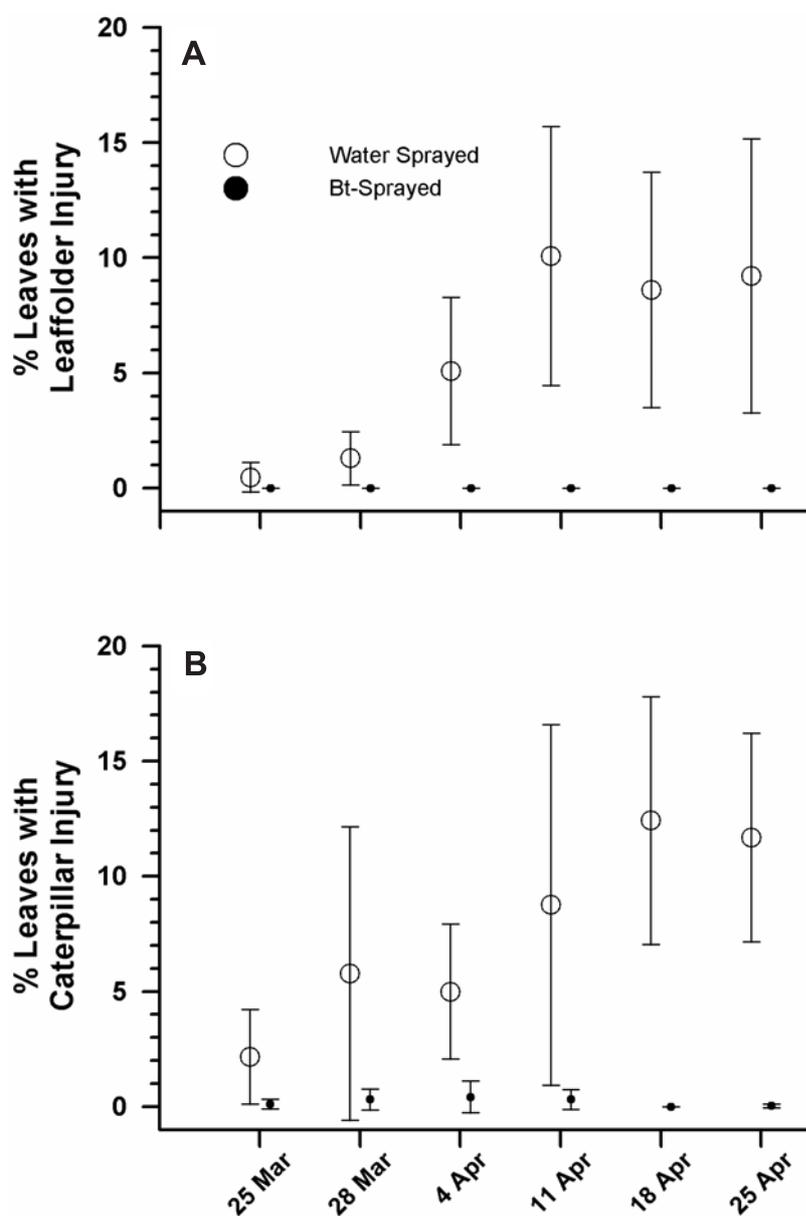
Of the two lepidopteran complexes scored for leaf injury, both revealed large treatment differences in pest injury to rice plants over all three sites (Fig. 1). Repeated application of *Bt* sprays widened the treatment effect for leaffolders and caterpillars over the growing season. Over the three sites, leaf injury inflicted by caterpillars was reduced significantly by *Bt* sprays on 28 March and 4 April (one-tailed *t*-tests = 12.005 and 4.434, *df* = 2, *P* < 0.05). For leaffolders, three marginally significant treatment differences were found (28 March, 11 April and 25 April). Site by site analysis revealed that water-sprayed plots in Sipit sustained the highest injury from herbivores (leaffolders and caterpillars), both in the

number and magnitude of significant differences (12 out of 12 cases), followed by Pila (11 out of 12 cases), and IRRI (8 out of 12). These and the other significant results above validated *Bt* sprays as a treatment variable in this experiment, at least for leaf-feeding lepidopterans. Stem borer damage did not differ between the *Bt* and water sprayed plots (data not shown). This result was expected, because stem borer larvae feed on the external surface of the plant only briefly, while penetrating the stem or leaf sheath to feed internally (Cohen et al., 2000). Although grain yields were not measured in this study, a large body of experimental data shows that defoliators rarely reduce yields in tropical rice ecosystems (*e.g.*, Heong et al., 1998), even when injury exceeds 15% of the total leaf number per plant as we observed in this study (Fig. 1).

### The fauna

In excess of 120 000 invertebrates were collected and identified from the three sites. The fauna was reduced to 14 838 and 22 645 members after terrestrial herbivores and their terrestrial natural enemies, respectively, were sorted, for a total pest-natural enemy fauna of 37 483 individuals (based on 22 sample dates). Although the floodwater fauna in irrigated rice fields can represent up to 90% of the total invertebrate abundance (Schoenly et al., 1998), the sampling method used in this study collected the terrestrial (canopy) component with greater efficiency and we therefore restricted the analysis to terrestrial species. This terrestrial collection represented 11 orders, 78 families, and 218 taxa (mostly genera and species).

Comparison of sites revealed high compositional similarities in herbivore and natural enemy taxa and in their ranked abundances. The two closest sites, IRRI and Sipit, were most taxonomically similar, sharing 62% of the taxa and 98.1% of the individuals, indicating that the more abundant taxa made up the joint taxa. IRRI and Pila were least taxonomically similar, sharing 56% of the taxa and 93% of the individuals. Among these joint taxa, abundance at one site was positively and significantly correlated with abundance at another for all pairwise comparisons of sites (range in  $r_s$ : 0.7124–0.7918, *P* < 0.01). Taken together, these results show that herbivore and natural enemy faunas at these sites are alike in several key respects, attributable, in large part, to proximity and standardized field methods (*e.g.*, sampling program, agronomic practices) that were followed across the sites.



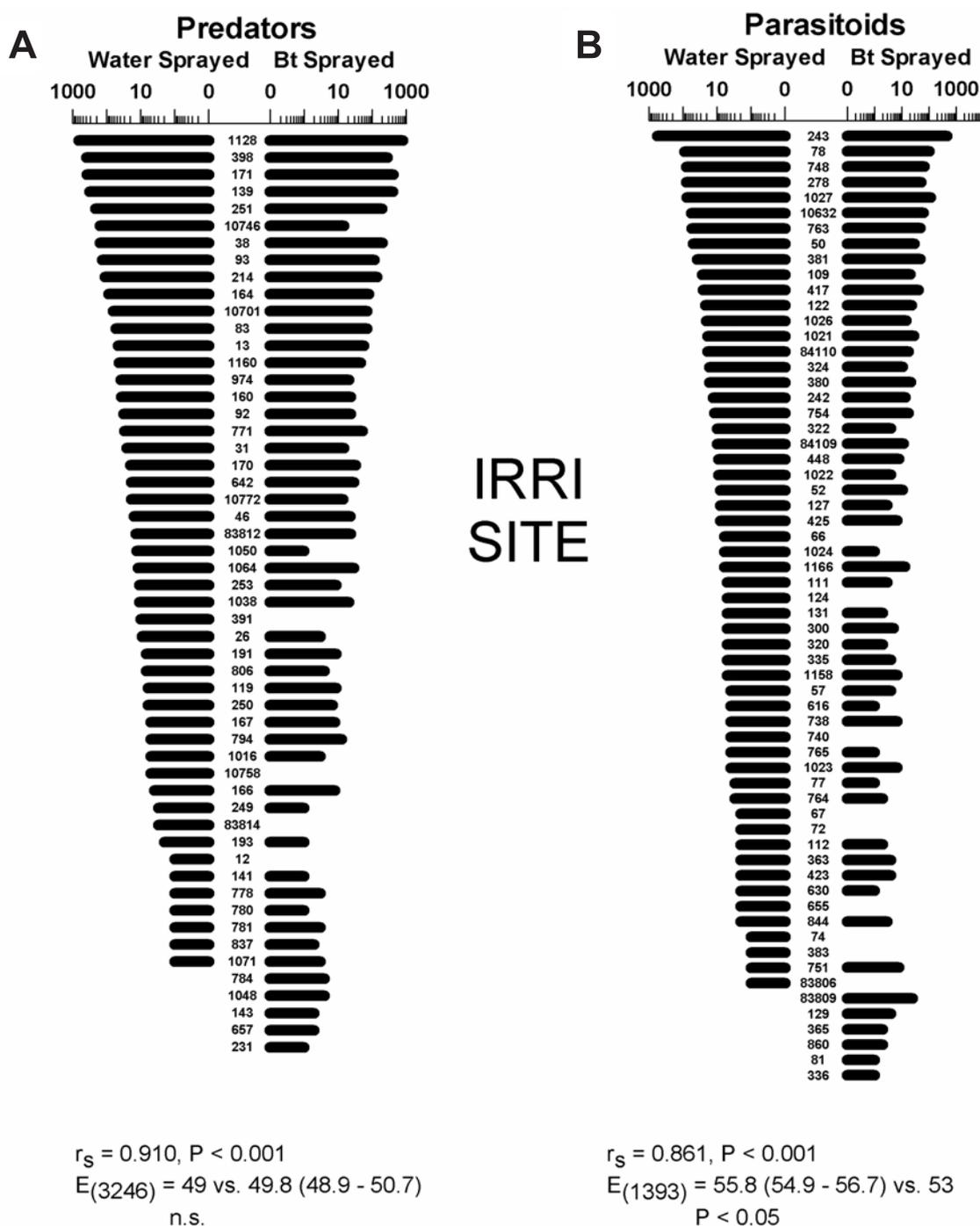
**Figure 1.** Leaf injury (mean  $\pm$  SE) caused by (A) leaffolders and (B) caterpillars on 10 randomly-chosen hills within water- and *Bt* sprayed plots for 6 assessment dates, averaged over the three sites.

### Do *Bt* sprays impact natural enemy complexes?

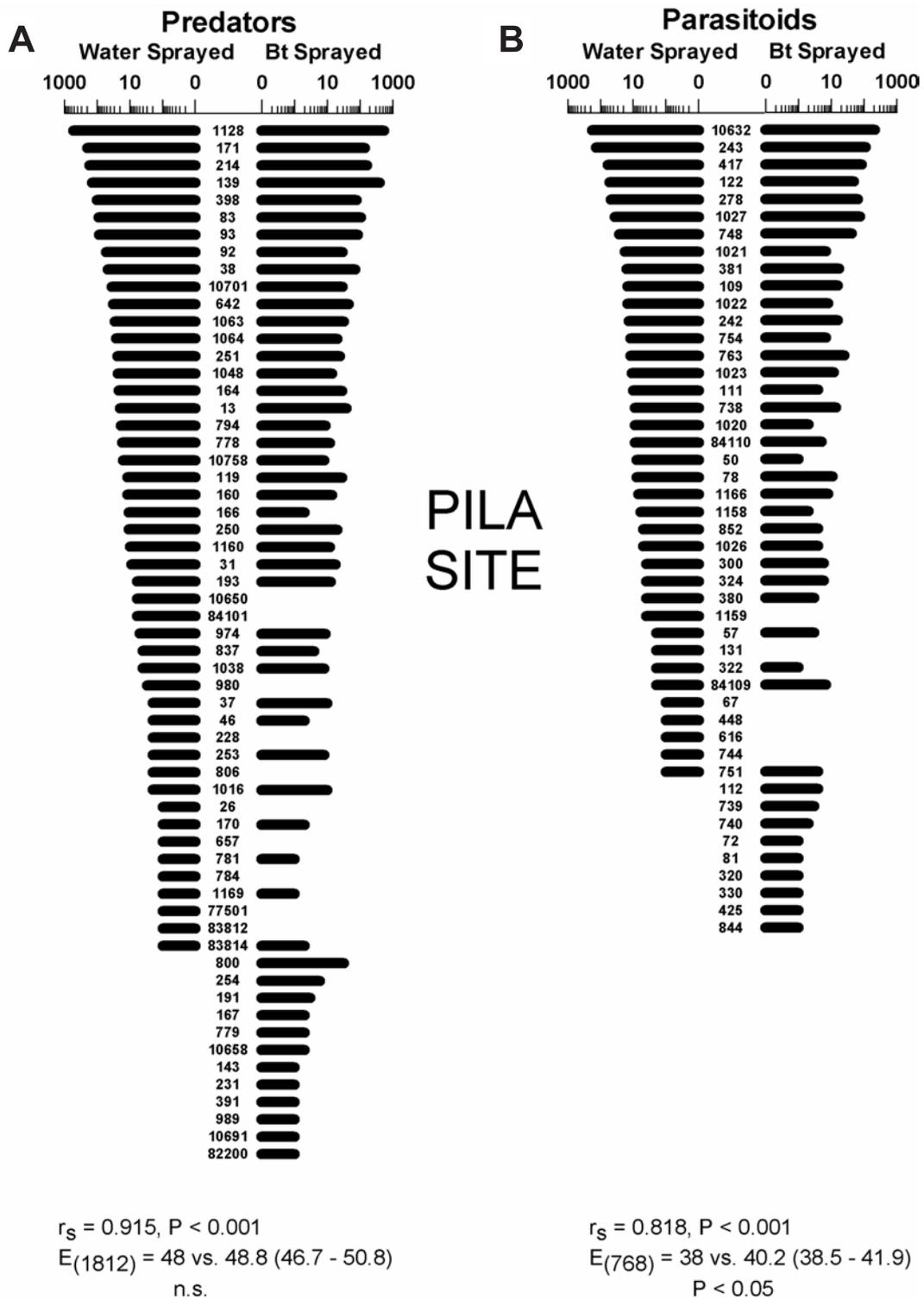
Whole complexes of predator and parasitoid faunas from each treatment and site fit a typical pattern of harboring a few common taxa ( $\geq 5\%$  of the total individuals), several intermediate (1–5%), and many rare taxa ( $< 1\%$ ), spanning nearly four orders of magnitude in abundance (Figs. 2–4). A range of 2–7 common taxa were collected from each site with most displaying wide distributions among sites. Abundant predatory taxa that were detected

in all sites, and treatments included Ceratopogonidae (taxon #1128, 6 out of 6 treatments; Figs. 2–4), *Cyrtorhinus lividipennis* Reuter (#171, 6 $\times$ ), and *Pardosa* sp. (#139, 6 $\times$ ). Among abundant parasitoids (and adult blood parasites), commonly reported taxa included *Gonatotocerus* sp. (#243, 6 out of 6), *Aedes* sp. (#10632, 6 $\times$ ), Scelionidae (#1027, 6 $\times$ ), and *Opius* sp. (#278, 6 $\times$ ). Matched rank abundance plots for water and *Bt* treatments show both strong and positive associations between treatments for predators and parasitoids, independent of site.

Non-target impacts of *Bt* sprayed rice

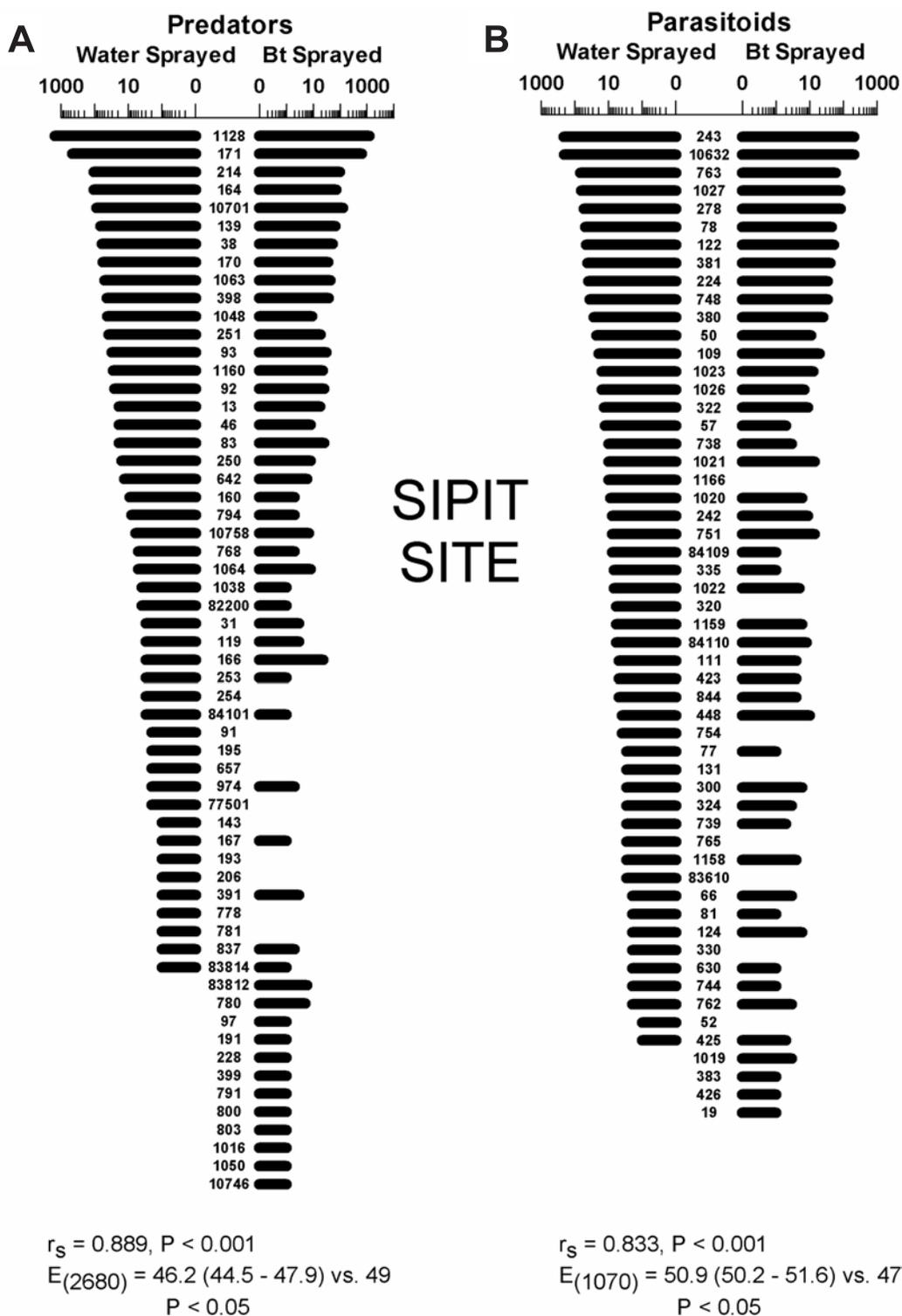


**Figure 2.** Matched rank-abundance plots for entire complexes of (A) predators and (B) parasitoids sampled from water- and *Bt* sprayed rice at IRRI during the dry season 1996. The left plot in each figure is the control treatment (water-sprays) against which the right plot (*Bt* treatment) is compared for analysis. Total invertebrate abundances are plotted on a logarithmic scale for readability. Taxon identification numbers are shown between the two treatments of each graph. The taxonomic identities of all natural enemy taxa are listed in the Appendix 1. The results of two ecostatistical tests are listed at the bottom: Spearman rank ( $r_s$ ) and the rarefaction metric,  $E(S_n)$ . Data for nymphs and adults were combined from 10 sample points, each having 2–4 hills, totaled across 22 sample dates.



**Figure 3.** Matched rank-abundance plots for entire complexes of (A) predators and (B) parasitoids sampled from water- and *Bt* sprayed rice at Pila during the dry season 1996. Panels A–B are as in Figure 2.

Non-target impacts of *Bt* sprayed rice



**Figure 4.** Matched rank-abundance plots for entire complexes of (A) predators and (B) parasitoids sampled from water- and *Bt* sprayed rice at Sipit during the dry season 1996. Panels A–B are as in Figure 2.

Rank abundance tests for all sites, as judged by Spearman ( $r_s$ ) correlation tests, show that catches from *Bt* and water sprays were significantly positively associated among their joint taxa. Most of the taxa that were common in one treatment were usually common in the other. Spearman rank statistics revealed that correlations, although significant at all sites, were somewhat stronger for predators than for parasitoids. After rarefying natural enemy abundance to a common size for each treatment pair, 4 of 6 treatment comparisons gave statistically different counts of predator and parasitoid taxa. In the two exceptional cases where comparable counts of taxa were found, both were predators (Figs. 2A and 3A). In summary, 8 of the 12 ecostatistical tests showed that *Bt* sprays had negligible impacts on overall natural enemy complexes, at least when tested for differences in species richness and abundance ranks.

Matched abundance plots also show that some abundant taxa, sampled from the water-sprayed plot, were found in lower numbers in the *Bt* plot. The most conspicuous examples are anthocorid bugs (#10746) at IRRRI in which 158 and 15 individuals were caught in water-sprayed and *Bt* plots, respectively. Other, less-noteworthy examples were: *Cotesia flavipes* Cameron (Braconidae: Hymenoptera, #57) at Sipit (13 and 2 individuals), *Ochthera* sp. (Ephydriidae: Diptera, #166) at Pila (11 and 2 individuals), and diapiiid wasps (#1020) at Pila (9 and 2 individuals). These differences, individually and in combination, however, were insufficient to change the otherwise strong and direct correlation in predator and parasitoid ranks.

Some taxa were sampled in such small numbers that they were caught in only one treatment. At IRRRI, for example, five predators (#s 784, 1048, 143, 657 and 231) and 6 parasitoids (#s 83809, 129, 365, 860, 81 and 336) were collected in relatively small numbers from the *Bt*-treated plot (range: 1–25 individuals) but went unsampled altogether in the water-sprayed plot. On balance, however, the number of “missing” taxa in the two treatments is nearly equal, with 48 on the water-sprayed side and 47 on the *Bt* side.

Temporal variation in mean abundance of predators and parasitoids is shown in Figures 5A and 5B. Predator and parasitoid populations increased steadily in both treatments with crop age, although they attained slightly higher densities in water-sprayed plots than *Bt* sprayed plots in the first half of the crop cycle. In the second half, predator and parasitoid densities in the *Bt* plots surpassed those of the control plots on approximately half the sampling dates. For predators and parasitoids, time-series analysis revealed that the pattern of *Bt* departures in mean

abundances from water-sprayed controls was random throughout the crop cycle (# runs ( $r$ ) for predators = 16,  $n_1 = 11$ ,  $n_2 = 11$ ,  $P > 0.05$ ;  $r$  for parasitoids = 8,  $n_1 = 12$ ,  $n_2 = 10$ ,  $P > 0.05$ ).

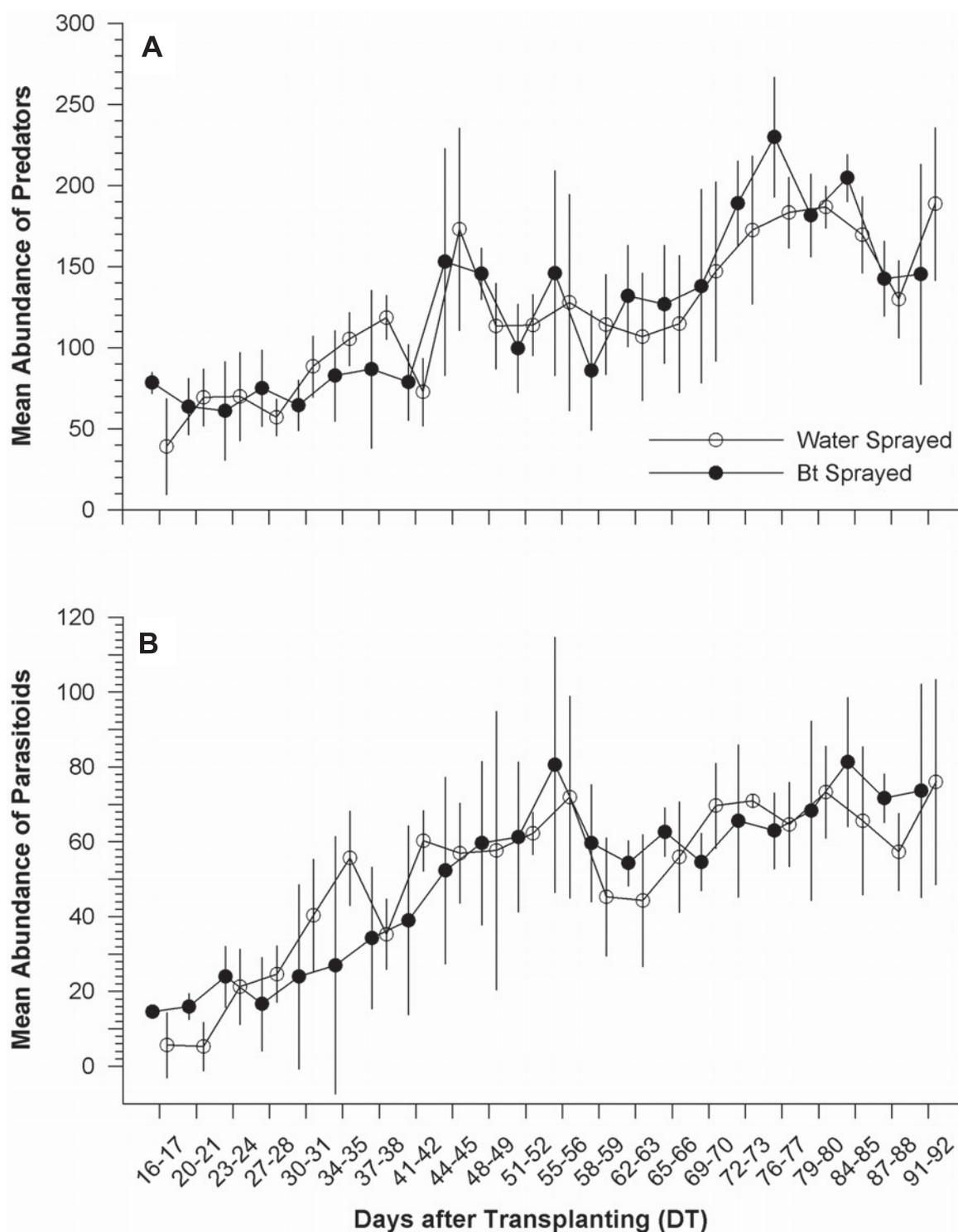
*Bt* sprays generally did not slow the accumulation rate of predator and parasitoid species over the growing season (Fig. 6). Within the first 40 days, *Bt* plots had accumulated predator and parasitoid species at a slightly faster rate than water-sprayed controls before the two treatments finally converged at mid season. In the last month of the crop season, accumulation rates were slightly faster in the control plots than *Bt* plots (Fig. 6).

### Do *Bt* sprays affect non-target herbivores?

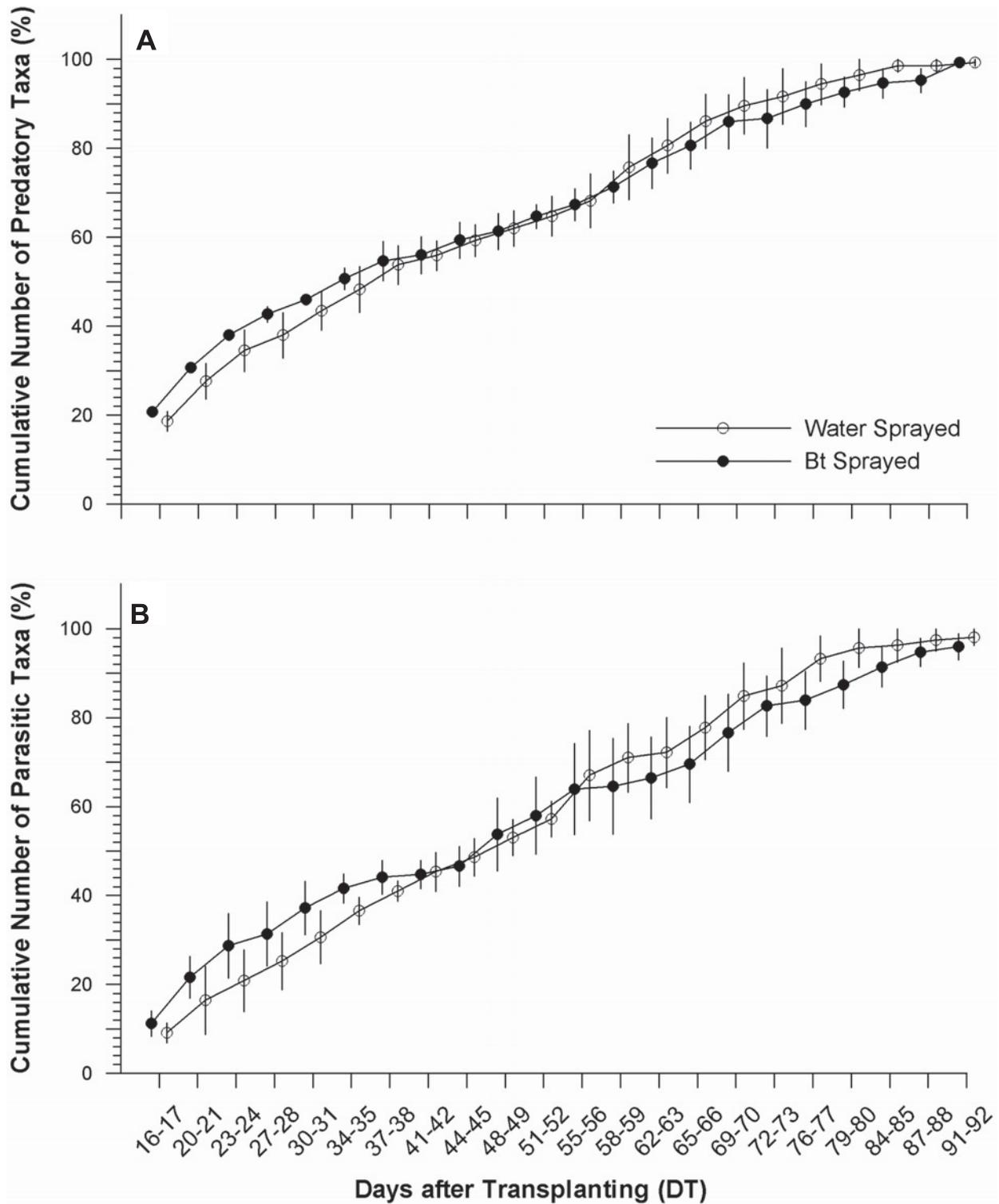
Due to their historical importance in tropical rice ecosystems, planthoppers (delphacid homopterans) and leafhoppers (cicadellid homopterans) are good candidates for testing non-target effects of *Bt* sprays and *Bt* plants. In this three-site study, three planthopper species were collected (*Nilaparvata lugens* [Stål], *Sogatella furcifera* [Horváth], and *Tagasodes pusanus* [Distant]) along with eight leafhopper species (*Cofana spectra* [Distant], *Nephotettix virescens* [Distant], *N. malayanus* Ishihara et Kawase, *N. nigropictus* [Stål], *Amrasca biguttula biguttula* [Ishida], *Balclutha* spp., *Nirvana philippinensis* Baker, and *Recilia dorsalis* [Motschulsky]).

For the three delphacids (Fig. 7A), mean population densities declined steadily with crop age in both treatments, displaying abundances 4–5 times larger at the start than the end of the crop season (Fig. 7A). Population trajectories of mean abundances for both treatments were very similar. The nonparametric runs test confirms that the pattern of departures in mean abundances was random ( $r = 10$ ,  $n_1 = 10$ ,  $n_2 = 12$ ,  $P > 0.05$ ). For the eight cicadellids, populations in both treatments rose steadily and then leveled off at mid season, though the *Bt* plot had slightly lower densities throughout this period (Fig. 7B). The runs test confirmed a lengthy (13-date) cluster of lower mean abundances in the *Bt* plots and showed a non-random pattern overall for cicadellids ( $r = 7$ ,  $n_1 = 17$ ,  $n_2 = 5$ ,  $P < 0.05$ ). These three-site results showed that populations of delphacids were unaffected by *Bt* sprays (Fig. 7A) and that cicadellids in both treatments were comparable in the second half of the crop season but were reduced in the first half in *Bt* sprayed plots compared to water-sprayed controls (Fig. 7B).

Non-target impacts of *Bt* sprayed rice

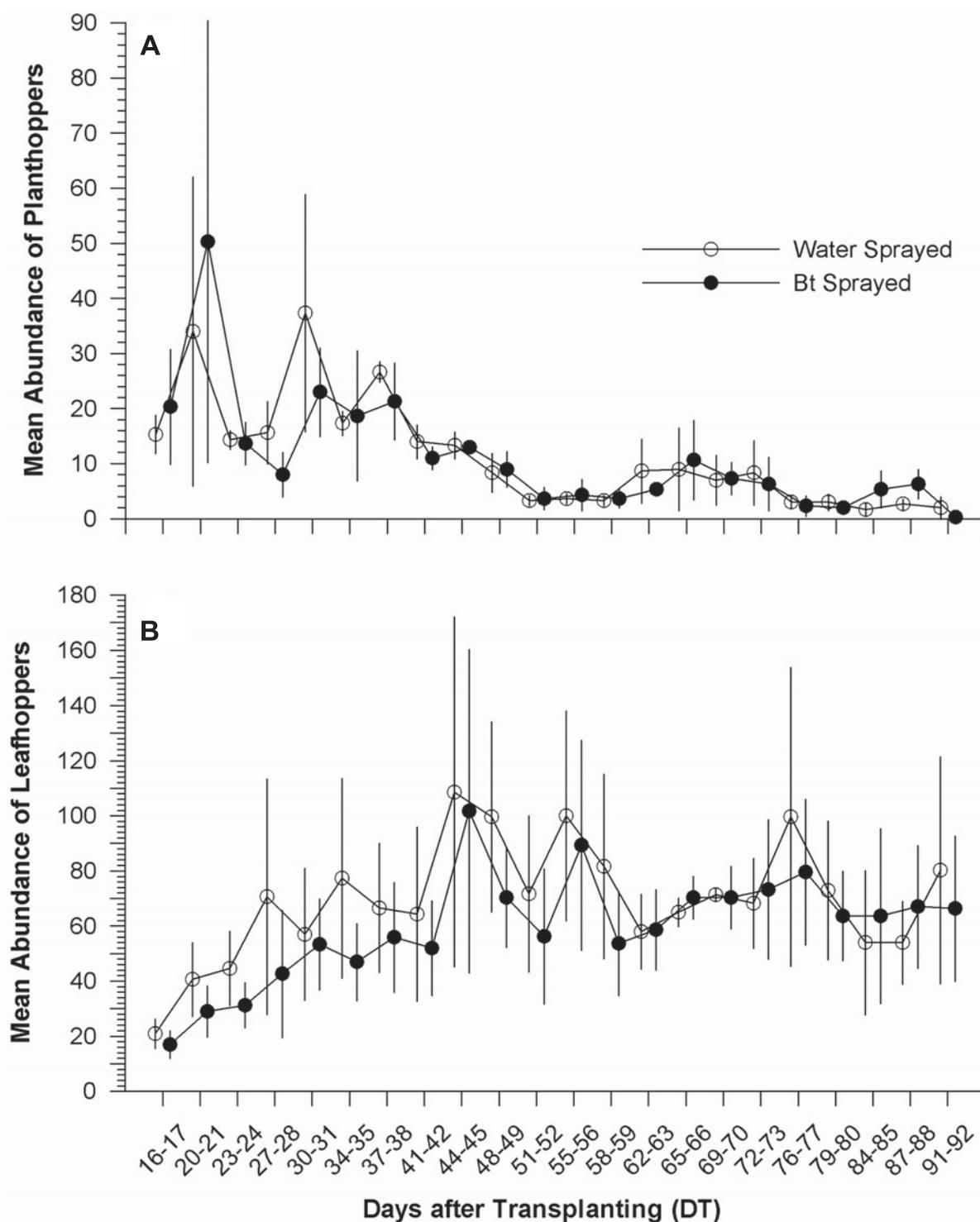


**Figure 5.** Counts (mean  $\pm$  SE) of (A) predators and (B) parasitoids sampled at three sites (IRRI, Pila, Sipit) from water- and *Bt* sprayed plots on each of 22 sampling dates during the dry season 1996. Data are numbers of nymphs and adults collected from 10 sample points, each having 2–4 rice hills per site.



**Figure 6.** Cumulative number (mean  $\pm$  SE) of (A) predatory and (B) parasitic arthropod taxa, averaged across the three sites, both as a function of time and normalized.

Non-target impacts of *Bt* sprayed rice



**Figure 7.** Counts (mean  $\pm$  SE) of (A) planthoppers and (B) leafhoppers sampled at three sites (IRRI, Pila, Sipit) from water- and *Bt* sprayed rice on each of 22 sampling dates during the dry season 1996. Data are numbers of nymphs and adults collected from 10 sample points, each having 2–4 rice hills per site.

**Table 1.** Guild-level properties of natural enemies of foliage-feeding lepidopteran herbivores in rice plots treated with water (control) and *Bt* sprays at three sites in the Philippines. The bioequivalence tests are based on mean abundances of natural enemies in each treatment, taken over the three sites (*i.e.*, average of three “I” values below).

Guild statistic <sup>1</sup>	Site and treatment					
	IRRI		Pila		Sipit	
	Water	<i>Bt</i>	Water	<i>Bt</i>	Water	<i>Bt</i>
S	6	7	9	10	8	5
I	120	78	167	210	137	149
E(S <sub>n</sub> ) and 95% CI	5.87 5.19–6.55	7*	9	9.17 7.56–10.78	8*	4.92 4.37–5.46
<i>r<sub>s</sub></i>	0.7701		0.9550**		0.9000	
<b>Bioequivalence test results<sup>2</sup></b>						
	Difference in mean abundance ( $\Delta_{crit}$ )	<i>t</i> -value	<i>P</i>			
	5	0.000	1.000			
	25	0.789	0.513			
	50	1.775	0.218			
	75	2.761	0.110			
	100	3.746	0.064			
	125	4.732	0.042			
	150	5.718	0.029			

<sup>1</sup> S = number of arthropod taxa; I = total (seasonal) abundance; E(S<sub>n</sub>) = rarefaction-adjusted species richness (S) with standardized samples in treatments to equal numbers of individuals (n); *r<sub>s</sub>* = nonparametric, tie-corrected (Spearman) rank correlations.

<sup>2</sup> H<sub>0</sub> =  $|\Delta_B - \Delta_W| \geq \Delta_{crit}$ , such that *Bt* sprays produce a socially-unacceptable effect ( $\Delta_{crit}$ ) on mean abundances of natural enemies of lepidopteran herbivores. Rejection of the null hypothesis implies that these data demonstrate that the observed difference is  $< \Delta_{crit}$ .

\* *P* < 0.05; \*\* *P* < 0.01.

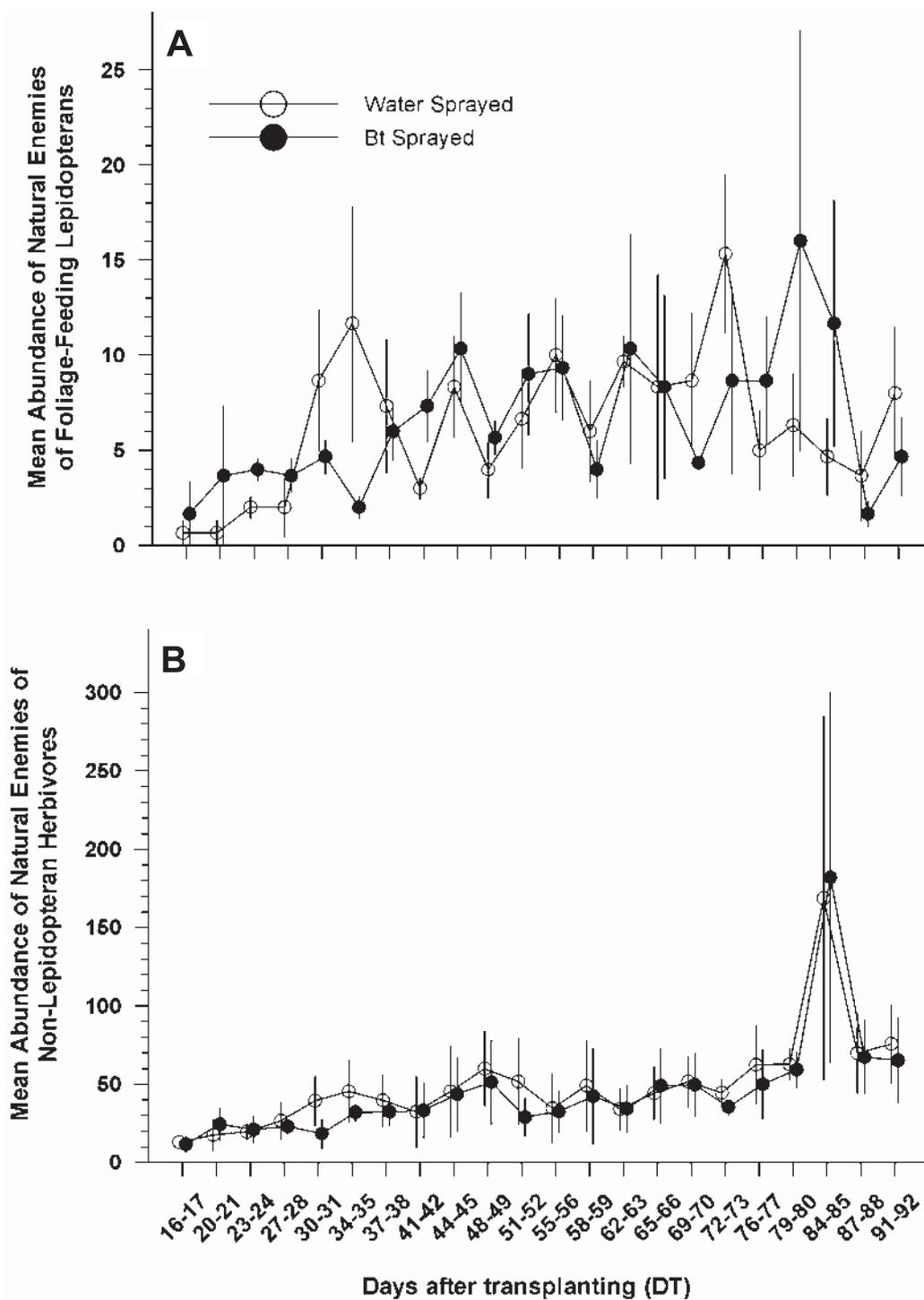
### Do *Bt* sprays impact natural enemies of foliage-feeding lepidopteran and non-lepidopteran herbivores?

Natural enemies of foliage-feeding lepidopterans (pyralids, noctuids, tineids, geometrids) constituted seven parasitoid taxa in six families (Braconidae, Elasmidae, Eulophidae, Encyrtidae, Bethylidae, Tachinidae) and two orders (Hymenoptera, Diptera). This guild had low membership, averaging only 7.5 taxa and 144 individuals per site. At IRRI and Sipit, significant treatment differences were evident in abundance ranks and rarefaction-adjusted species richness (Tab. 1). Despite these differences, however, mean abundances in control plots (3-site mean = 141 individuals per season, SE = 13.1, 95% CI = 84–197) and *Bt* plots (mean = 146 individuals per season, SE = 38.1, 95% CI = 0–310) were statistically

indistinguishable ( $t = 0.20$ ,  $df = 2$ ,  $P > 0.05$ ). One-way bioequivalence tests revealed that, although these treatment means differed by a small amount ( $\Delta_{obs} = 5$  individuals per season), the true difference between treatments is unlikely to exceed 125 individuals per season ( $\Delta_{crit}$ ) at the 0.05 level (Tab. 1). Time-series plots showed that mean abundances of natural enemies of foliage-feeding lepidopterans were higher in *Bt* plots than control plots in more than half the sampling dates; however, on nine sampling dates mean abundances were 1.1 to 5.8 times higher in water-sprayed plots than *Bt* plots (Fig. 8A). The non-parametric runs test showed that the pattern of departures in mean abundances in the *Bt* plots was random ( $r = 7$ ,  $n_1 = 9$ ,  $n_2 = 12$ ,  $P > 0.05$ ).

Natural enemies of non-lepidopteran herbivores (*i.e.*, homopterans, hemipterans, dipterans) constituted a large guild of 20 predator and parasitoid taxa distributed over

Non-target impacts of *Bt* sprayed rice



**Figure 8.** Counts (mean  $\pm$  SE) of (A) natural enemies of foliage-feeding lepidopteran herbivores, and (B) natural enemies of non-lepidopteran herbivores (hemipterans, homopterans, and dipterans) and non-herbivores sampled from three sites from water- and *Bt* sprayed rice on each of 22 sampling dates during the dry season 1996. Data are numbers of adults collected from 10 sample points, each having 2–4 rice hills per site.

**Table 2.** Guild-level properties of natural enemies of non-lepidopteran (homopteran, hemipteran and dipteran) herbivores in rice plots treated with water (control) and *Bt* sprays at three sites in the Philippines. The bioequivalence tests are based on mean abundances of natural enemies in each treatment, taken over the three sites (*i.e.*, average of three “I” values below).

Guild statistic <sup>1</sup>	Site and treatment					
	IRRI		Pila		Sipit	
	Water	<i>Bt</i>	Water	<i>Bt</i>	Water	<i>Bt</i>
S	26	24	18	19	22	25
I	1968	1787	593	543	703	632
E(S <sub>n</sub> ) and 95% CI	25.9 25.3–26.5	24*	17.7 16.7–18.7	19*	21.6 20.4–22.8	25*
<i>r<sub>s</sub></i>	0.9655**		0.9311**		0.8727**	
<b>Bioequivalence test results<sup>2</sup></b>						
	Difference in mean abundance ( $\Delta_{crit}$ )	<i>t</i> -value	<i>P</i>			
	100	0.299	0.793			
	150	1.059	0.401			
	200	2.417	0.137			
	250	3.774	0.064			
	300	5.132	0.036			
	350	6.490	0.023			

<sup>1</sup> S = number of taxa; I = total (seasonal) abundance; E(S<sub>n</sub>) = rarefaction-adjusted species richness (S) with standardized samples in treatments to equal number of individuals (n); *r<sub>s</sub>* = nonparametric, tie-corrected (Spearman) rank correlations.

<sup>2</sup> The null hypothesis is:  $|\Delta_B - \Delta_W| \geq \Delta_{crit}$ , such that *Bt* sprays produce a socially-unacceptable effect ( $\Delta_{crit}$ ) in mean abundances of natural enemies of non-lepidopteran herbivores. Rejection of the null hypothesis implies that these data demonstrate that the observed difference is  $< \Delta_{crit}$ .

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

18 families and seven orders. Significant treatment differences were evident in rarefaction-adjusted species richness at each site, however, Spearman rank tests showed statistically positive correlations in abundance ranks in each treatment pair, regardless of site (Tab. 2). Despite these differences in species richness, mean abundances in control plots (3-site mean = 1103 individuals per season, SE = 434.7, 95% CI = 0–2972) and *Bt* plots (mean = 992 individuals per season, SE = 400, 95% CI = 0–2712) were statistically comparable ( $t = 3.01$ ,  $df = 2$ ,  $P > 0.05$ ). Bioequivalence tests revealed that, although these treatment means differed by a moderate amount ( $\Delta_{obs} = 111$  individuals per season), the true difference between treatments is unlikely to exceed 300 individuals per season ( $\Delta_{crit}$ ) at the 0.05 level (Tab. 2). Population trajectories of mean abundances were nearly identical for both treatments over the cropping cycle (Fig. 8B) and the nonparametric runs test confirmed that the pattern of departures in mean abundances was random ( $r = 9$ ,

$n_1 = 16$ ,  $n_2 = 5$ ,  $P > 0.05$ ). The late season spike in natural enemies in both plots, occurring mostly at IRRI, was largely due to linyphiid (*Atypena formosana* [Oi]) and theridiid (*Theridion* sp.) spiders (Fig. 8B) whose numbers normally rise over the season (Schoenly et al., 1996a; Settle et al., 1996) in fields not treated with insecticides.

### Do *Bt* sprays reshape rice field trophic structure?

Spores from *Bt* sprays represent an additional food source that may increase community productivity. Such “bottom-up effects” are predicted to increase abundances of carnivores, but not herbivores (Morin, 1999). In this analysis, web taxa and their abundances that were previously sorted by trophic level were totaled, then averaged over the 3 sites. Site by site analysis of trophic structure yielded 8 trophic levels for IRRI and 6 each for

## Non-target impacts of *Bt* sprayed rice

**Table 3.** Total (seasonal) abundances of invertebrates by trophic level in water- and *Bt* sprayed rice, averaged over three sites in the Philippines, based on 22 sample dates. Trophic levels 3–6 include taxa found in the Philippines-wide web.

Trophic level <sup>1</sup>	Means ± SD		95% CI for mean		<i>t</i> -test
	Water	<i>Bt</i>	Water	<i>Bt</i>	
2	1899.3 ± 471.4	1807.3 ± 30.7	729.0–3069.7	1731.2–1883.5	–0.327
3	1283.3 ± 605.6	1301.7 ± 558.2	0–2786.8	0–2687.5	0.434
4	92.0 ± 67.2	79.7 ± 59.7	0–258.8	0–227.8	–2.565
5	655.3 ± 379.0	729.7 ± 414.1	0–1596.2	0–1757.6	0.933
6	54.7 ± 27.8	64.3 ± 38.9	0–123.7	0–160.9	1.096

<sup>1</sup> Taxa were separated into trophic levels using the long-way-up algorithm (see methods: invertebrate identifications, feeding guilds, and web construction).

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

Pila and Sipit and, except for the fifth trophic level, mean abundances followed a typical pyramidal progression (Tab. 3). Despite a greater mean abundance in *Bt* plots than controls in 3 out of 5 trophic levels, wide-ranging confidence intervals and one-sided *t*-tests failed to show that *Bt* sprays significantly impacted trophic structure.

## DISCUSSION

The application of *Bt* sprays removed foliage-feeding lepidopteran larvae from the treated rice plots, as indicated by the near complete lack of herbivory (Fig. 1). Two ecostatistical tests revealed that removal of these larvae, as would occur in fields of *Bt* rice, had negligible effects on whole communities of other organisms, including predators and parasitoids not susceptible to *Bt* toxins. In the few cases where significant treatment differences were found (Figs. 2–4, Tabs. 1 and 2), tests showed that *Bt* sprays altered up to two aspects of community or guild structure, *i.e.*, species richness and abundance ranks; however, these differences were insufficient to affect population trajectories (Fig. 5), accumulation rates (Fig. 6) or mean abundances (Fig. 8), suggesting that *Bt* impacts were, at most, minor. The use of food web data to sort out different subsets of natural enemies revealed complex impacts in this agroecosystem while focusing attention on the ecological services these species provide (Losey et al., 2002).

Whole-community tests showed that in the four cases where significant treatment differences were found, *Bt* sprays altered parasitoid richness at 3 of 3 sites and predator richness at 1 of 3 sites; however, in half of these cases *Bt* plots had higher species richness (Figs. 2–4).

Nonparametric (Spearman) tests on ranked abundances showed that correlations, although significantly positive between all treatment pairs, were stronger for predators than for parasitoids. Taken together, these results suggest that parasitoid complexes may have been more sensitive than predators to the effects of *Bt* sprays.

The guild-level results for natural enemies suggest that *Bt* toxins directed against lepidopteran larvae would have negligible impacts on predators and parasitoids in tropical rice. Population trajectories of natural enemies of foliage-feeding lepidopterans revealed no treatment effect, though there were substantial fluctuations in mean abundances (Fig. 8A). The early and sustained increases in natural enemies of non-lepidopteran herbivores (Fig. 8B) coincided with large populations of alternative prey (*e.g.* detritivorous insects) in both treatments, as hypothesized by Settle et al. (1996), followed by planthoppers (Fig. 7A), leafhoppers (Fig. 7B) and non-herbivorous prey. By contrast, broad-spectrum insecticides have been shown to have deleterious effects on beneficial organisms. When chlorpyrifos, an organophosphate, was applied early (29 DT) in tropical rice ecosystems, the rank order and abundances of the five most common predators shifted chaotically while the five most common herbivores changed little (Heong and Schoenly, 1998; K.G.S., unpublished data). Likewise, Settle et al. (1996) showed that multiple, early (15–37 DT) sprays of carbofuran and monocrotophos in Indonesian rice fields disrupted biological control by reducing and delaying development of surface-dwelling predators on the plant and water surfaces.

Likewise, our species accumulation curves and time-series analyses revealed little evidence that *Bt* sprays altered either the buildup or the trajectories of non-target

herbivores or predators and parasitoids over the growing season (Figs. 5–7). Indeed, except for leafhoppers (Fig. 7B), both treatments produced remarkably similar population trajectories, magnitudes of variation, and peak behavior in abundance, regardless of functional group. Analysis of guild-level trajectories also revealed population behavior and treatment differences that went undetected in whole-community studies of predators and parasitoids. Ecostatistical results showed that the *Bt* and water sprays were more similar at the community level (67% of cases, Figs. 2–4) than at the guild level (42%, Tabs. 1 and 2, Fig. 8).

Overall, the guild-level results did not support the hypothesis that reduction in foliage-feeding lepidopterans caused by *Bt* sprays could lead to reduced populations of natural enemies and consequently increased populations of non-target herbivores. Abundances of natural enemies of non-lepidopteran herbivores in both treatments were remarkably similar throughout the growing season; the sharp peak in mean abundance in both plots late in the season (Fig. 8B) suggests that natural enemies may have responded to rising levels of leafhoppers (Fig. 7B). Leafhopper populations were smaller in the *Bt* sprayed plots during the first half of the season (Fig. 7B), possibly because some polyphagous natural enemies switched to detritivorous or leafhopper prey due to the absence of foliage-feeding lepidopteran larvae.

In the one-way bioequivalence tests (Tabs. 1 and 2), several increasingly liberal thresholds were presented representing alternative hypotheses to the null hypothesis of nonequivalence in mean abundances of natural enemies that take lepidopteran and non-lepidopteran herbivores in their diet or host range (Tabs. 1 and 2). The wide acceptance region for the null hypothesis in both cases (*i.e.*, 75 and 200 individuals per season, Tabs. 1 and 2) reflects the low precision that accompanied our small sample sizes (Dixon and Garrett, 1994). In the context of *Bt* sprayed rice in tropical Asia, if regulatory agencies set  $\Delta_{\text{crit}} = 125$  individuals per season for natural enemies of foliage-feeding lepidopterans and 300 individuals per season for natural enemies of foliage-feeding homopterans, hemipterans and dipterans, then any difference less than 125 and 300 individuals, respectively, would be considered insignificant to society and the null hypothesis of nonequivalence would be rejected. Conversely, if regulatory agencies chose smaller  $\Delta_{\text{crit}}$  values for these variables, the data would fail to demonstrate that *Bt* sprays affected natural enemies by a small amount.

*Bt* sprays are an imperfect substitute for *Bt* plants in that they do not control lepidopterous stem borers, the principal targets of *Bt* rice. However, the failure of *Bt*

sprays to control stem borer larvae would probably not result in large differences between the community of predators and parasitoids in *Bt* sprayed plots and plots of *Bt* rice. Stem borer larvae spend only short periods of time outside of the rice plant. Newly eclosed larvae enter the rice plant within 1–12 h after eclosion from the egg. Later in development, most larvae move once or twice to a new tiller (Cohen et al., 2000), presumably as the food quality of the old tillers decline. The egg masses and neonate larvae would be available to predators (*i.e.*, tettigoniids, and gryllids) and parasitoids (*i.e.*, trichogrammatid, scelionid, and eulopid wasps) (Reissig et al., 1986) in both *Bt* sprayed plots and plots of *Bt* rice. Older larvae and pupae are protected from predators within the stem, with the exception of the episodes of movement noted above. Larvae and pupae within stems are attacked by braconid, ichneumonid, and elasmid parasitoids (Reissig et al., 1986). Our three-site food webs show that these wasps are lepidopteran specialists on stem borers (*Telenomus rowani* [Gahan] [Scelionidae], taxon #52; *Amauromorpha accepta metathoracica* [Ashmead] [Ichneumonidae], #66; see Figs. 2 and 4), stem borers and leafhoppers (*Temelucha philippinensis* [Ashmead] [Ichneumonidae], #72; *Charops brachypterum* Maheswary and Gupta [Ichneumonidae], #67; and *Trichomma cnaphalocrosis* Uchida [Ichneumonidae], #74; Figs. 2 and 3) and on stem borers and noctuids (*Cotesia flavipes* Cameron [Braconidae], #57; Figs. 2 and 4). Their reduced abundance in plots of *Bt* rice should therefore have limited impact on other species. In this study, their mean abundances in control plots (3-site mean = 14 individuals per season, SE = 5.9, 95% CI = 0–39) and *Bt* plots (mean = 8 individuals per season, SE = 3.5, 95% CI = 0–23) were statistically indistinguishable ( $t = 1.66$ ,  $df = 2$ ,  $P > 0.05$ ). This situation may also be analogous for two hyperparasitoids (*Trichomalopsis apanteloctenae* Crawford [Pteromalidae], taxon #50 and *Tetrastichus* spp. [Eulophidae], #78) that were collected at all three sites (Figs. 2–4). The pteromalid parasitizes *T. rowani* while the eulophid parasitizes *T. philippinensis*, two parasitoids of stem borers, and a few other hosts including leafhoppers and stem borers. In this study, their mean abundances in control plots (3-site mean = 77 individuals per season, SE = 36.4, 95% CI = 0–234) and *Bt* plots (mean = 67 individuals per season, SE = 35.3, 95% CI = 0–219) were also statistically comparable ( $t = 2.78$ ,  $df = 2$ ,  $P > 0.05$ ). The failure of this study to reveal significant impacts of *Bt* technology on (these and other) terrestrial species does not preclude the possibility that *Bt* sprays or *Bt* rice may significantly alter the floodwater

or benthic fauna. Ecosystem-level responses that link the terrestrial and floodwater portions of irrigated rice fields (Schoenly et al., 1998; Settle et al., 1996; Simpson et al., 1993a; 1993b) and their effects on rice productivity (Simpson et al., 1994a; 1994b) remain poorly understood.

Laboratory and field studies of selected arthropod species have also provided evidence that *Bt* rice will not disrupt biological control of non-target herbivores. Bernal et al. (2002) tested five *Bt* rice lines, each with a different toxin gene promoter, and found no toxicity of the plants to the brown planthopper and no negative effects on *Cyrtorhinus lividipennis* (Hemiptera: Miridae) that preyed on brown planthoppers reared on *Bt* rice. In a field study in China, Liu et al. (2002) found no significant differences in adult densities of *Nephotettix cincticeps* and the whitebacked planthopper, *Sogatella furcifera*, between plots of two *Bt* rice lines and a control line, although densities of nymphs of both species were higher at some sample dates early in the season on one of the *Bt* lines. Densities of five common spiders were similar between the *Bt* and control lines.

While our results provide an indication that *Bt* rice is unlikely to disrupt the biological control of non-target herbivores such as planthoppers and leafhoppers, there are reasons why these results should be interpreted with caution. First, the partial resistance of the rice varieties used (IR36 and IR64) to two important non-target herbivores (the homopterans *N. lugens* and *N. virescens*) may have prevented population increases of these species that would otherwise have occurred due to possible disruption of biological control. Experiments with rice varieties that are susceptible to these homopterans would be necessary to address this question. Second, populations of rice leafhoppers tend to be low in the Philippines (De Kraker et al., 1999). Elimination of leafhopper larvae by *Bt* sprays or *Bt* rice might have a larger effect on the overall arthropod community in areas where leafhoppers are generally more abundant. Third, as noted above, *Bt* sprays do not control stem borer larvae and thus do not fully simulate the effects of *Bt* plants on the arthropod community. Experiments in actual *Bt* rice fields are clearly necessary. Fourth, *Bt* sprays result in the deposition of materials not present in *Bt* plants, such as *Bt* spores and inert formulation ingredients. Although recovery of *Bt* spores deposited on foliage and in soil declines rapidly (Petras and Casida, 1985; West and Burges, 1985), we applied sprays twice each week throughout the experiment. Nonetheless, this press-type manipulation (Bender et al., 1984) did not result in significant bottom-up effects attributable to the *Bt* spores

(Tab. 3). Finally, it is likely that immigration into our *Bt* sprayed plots accounted in part for the lack of differences in the arthropod community between the *Bt*- and water-sprayed plots. In areas where *Bt* rice cultivars, once released, are widely adopted by farmers there will be few non-*Bt* rice fields to serve as sources of immigrating arthropods into *Bt* rice fields, particularly natural enemies of lepidopterous stem borers and foliage feeders. This may result in further changes in the arthropod community that did not occur in our 0.25-ha *Bt* sprayed plots.

Field testing of *Bt* rice in Asia will probably remain limited for several years, and in those countries where field tests may occur, plot sizes of initial tests will likely be small. For example, the individual plot sizes in early field tests of *Bt* rice in China were 5–13 m<sup>2</sup> (Tu et al., 2000), 8–42 m<sup>2</sup> (Ye et al., 2001), and 333 m<sup>2</sup> (Liu et al., 2002). Such plots are too small for meaningful studies of the effects of *Bt* rice on population dynamics of non-target arthropods. Given these limitations, further experiments using *Bt* sprays to investigate possible effects of *Bt* rice on predators, parasitoids, and the biological control of non-target pests may be worthwhile.

Our study of three fields within one of Asia's largest "rice bowls" did not share the same problems of scale and replication of other ecosystem-level studies (Carpenter, 1988). Unlike some whole-lake studies that went unreplicated because potential sites differed ecologically (Carpenter, 1990; Schindler, 1998), our sites were similar enough to be considered replicates because the three fields were within a 15-km radius, followed the same agronomic and experimental practices, and had high compositional overlap of the same abundant taxa. Survey data on rice farms from eight selected Asian countries, including the Philippines, reveals that 57% of the rice farms surveyed between 1982 and 1991 were ≤ 1 ha in size (IRRI, 1994), justifying our use of 0.5-ha sized fields in this study. When an extensive food web is available, such as that for rice in the Philippines, and when it is combined with matched ranked-abundance plots, time-series comparisons and ecostatistical tests, these methodologies provide a sensitive comparison of control and *Bt*-treated fields that can be applied to future studies of *Bt* sprays or *Bt* crops. Taken together, these features increased the ecological realism of the experiment, at least when compared to laboratory and single-population studies. Nevertheless, and to underscore the need for more field experiments, a more conclusive test of the impact of *Bt* rice will require experiments with transgenic plants, conducted in a range of Asian environments, and over multiple cropping seasons.

## MATERIALS AND METHODS

### Study sites and field preparation

The experiment was conducted at three sites in Laguna Province, Philippines, in the 1996 dry season (Jan-May). The three sites were within a 15-km radius. One was on the IRRI Experiment Station and two (Pila and Sipit) were in farmers' fields. Both the IRRI and Sipit sites were in the town of Bay, and the Pila site was in the town of Pila. At each site there were two adjacent 0.25 ha plots separated by earthen bunds. The treatments were replicated across sites but not within sites. One plot was sprayed twice weekly with a commercial *Bt* formulation while the second was sprayed with water (details in the following section). Total field sizes at each site (0.5 ha) mirror the majority (57%) of rice farms in many Asian countries (IRRI, 1994).

The plots of each site were fertilized with 50 kg ha<sup>-1</sup> of ammonium sulfate at the time of land preparation and received an additional 50 kg ha<sup>-1</sup> at both the maximum tillering and panicle initiation stages. Plots were treated with 750 g (active ingredient) ha<sup>-1</sup> butachlor herbicide and 250 g a.i. ha<sup>-1</sup> niclosamide for control of the golden apple snail (*Pomacea* sp.) approximately one-week before transplanting. Twenty-one-day-old rice seedlings were transplanted at 20 × 20 cm spacing.

### Experimental design

The plots were sprayed every 3 or 4 days, beginning at ≈2 week after transplanting and continuing until ≈1 week before harvest. Although the toxicity of *Bt* spores declines rapidly on foliage (Pinnock et al., 1971), the twice-weekly spray frequency insured a continual resupply on the rice plants. On each spray date, the *Bt* plots were treated with 1 kg ha<sup>-1</sup> foliar spray of a *B.t. aizawai* formulation (XenTari® Abbott Laboratories, North Chicago IL) in a water volume of 64 L ha<sup>-1</sup>. Our laboratory experiments have shown that a *B.t. aizawai* product is more effective against leaffolders than a *B.t. kurstaki* product (B.G., unpublished data). The control plots were treated with 64 L ha<sup>-1</sup> of water. Sprays were applied with hand-operated backpack sprayers.

Samples were collected according to a stratified random design. Each plot was divided into 10 approximately equal units and one sample was collected from a randomly chosen location within each unit on each sampling date. The sample location was marked with a bamboo

stake that was left in place for the next four sample dates, during which time the marked location was not resampled.

### Field sampling methods

The invertebrate fauna of the plots was sampled every 3 or 4 days between 0700 and 1000 h and the sprays were applied immediately following completion of sampling. Thus, the samples taken on the first sample date were collected before any sprays had been applied. Sensitivity tests of this collecting method reveal that early morning samples (0730–0930) harbor more invertebrate taxa than those taken later in the day (Schoenly and Zhang, 1999b). The 3-h sampling window prevented all three sites from being sampled on the same day; consequently, the Pila and Sipit sites were regularly sampled 1 day later than the IRRI site. For graphing purposes, sample dates were represented as 2-d intervals rather than a single day.

The samples were collected with a vacuum sampling device similar to that described by Domingo and Schoenly (1998), except that the sampling enclosure was a box with a metal frame and sides made of Mylar film. The box was 75 cm high and had openings of 50 × 50 cm at the base and 38 × 38 cm at the top. The enclosure was placed over four hills at a time during the vegetative growth stage (seedling germination to panicle initiation, 50 days or more) and two hills at a time during the reproductive (panicle initiation to booting to flowering, ca. 35 days) and ripening (flowering to grain maturity and harvest, ca. 30 days) stages. The top enclosure was open. Therefore, some strong flying, easily disturbed insects such as dragonflies may have escaped before being collected by the vacuum. Because species richness and abundance of rice-invertebrate populations increase with crop age (*e.g.*, Schoenly et al., 1998), invertebrates were collected from the rice foliage and water surface for ≈1 min during the vegetative growth stage, gradually increasing up to ≈3 min at the ripening stage. Performance tests of our suction sampler show that 85, 75 and 62% of the taxa are captured in the first min at the vegetative, reproductive and ripening stages, respectively, when tested over a 5-min continuous sampling interval. By the end of two min at the reproductive stage and 2.5 min at the ripening stage, the percentages of taxa (88% for both) are nearly equal to that of the vegetative stage at one min (K.G.S., unpublished data). Sampling intensity is also known to influence qualitative descriptors of food webs (Bersier et al., 1999; Goldwasser and Roughgarden, 1997; Martinez et al., 1999). For example, Bersier et al. (1999)

showed that low sampling effort can produce the appearance of scale invariance in intrinsically scale-dependent systems. Thus, increases in sampling duration over the cropping season were necessary to compensate for differences in arthropod richness and abundance over time and to insure comparability of samples in both time-series and cumulative tests.

### **Invertebrate identifications, feeding guilds, and web construction**

All samples were stored in 70% ethanol and sorted under a dissecting microscope. Specimens were identified by two parataxonomists who received 2 week (80 h) prior training in invertebrate taxonomy by one of us (A.T.B.). Organisms were sorted to stage (immatures and adults) then identified to taxon. Easily identified invertebrates were identified to species; less familiar or more difficult taxa were identified only to genus or family. Final taxonomic identities were checked against the invertebrate reference collections housed in the Taxonomy Laboratory of the IRRI Entomology and Plant Pathology Division. (Voucher specimens from this study were deposited in the same unit.)

In this study we focused on canopy-only (terrestrial) herbivores (feeders on rice, aquatic plants) and natural enemies of herbivores (predators and parasitoids). Non-terrestrial taxa (*e.g.* aquatic and benthic taxa) were eliminated from computerized spreadsheets prior to data analysis. Guild assignments of each taxon (adult and immature) followed the system of Schoenly *et al.* (1998) and Schoenly and Zhang (1999a) based on field observations of feeding behavior, mouthpart morphology, and literature sources dealing with close relatives.

Webs representing each of the three sites were constructed using the Philippines-wide food web (Cohen *et al.*, 1994) with all taxa from each site that matched those in the Philippines-wide web included. The Philippines-wide web has 546 rice-associated taxa and includes multiple classes of functional groups: 37 herbivores, 293 predators, 165 parasitoids, 14 parasites, 11 omnivores, and 25 pathogens. Trophic links were determined from field observations, field-exposing eggs of potential host species to collect immature parasitoids and parasites, predator preference and selectivity tests in the laboratory, and vertebrate stomach-content analysis. As in earlier food web studies (Cohen *et al.*, 1994; Schoenly and Cohen, 1991; Schoenly *et al.*, 1996a; 1996b), we assumed that enemy A ate or parasitized resource B at the site if and only if one life stage of A eats or parasitizes at least one life stage of B in the Philippine web, and taxon

A and B occurred at this site. Using the long-way-up algorithm of Cohen and Luczak (1992), we then separated known enemies of each herbivore (predators and parasitoids) and sorted natural enemies into trophic levels. Predators and parasitoids that included only foliage-feeding lepidopterans (*i.e.*, pyralids, noctuids, tineids, and geometrids) in their diet and host range were designated "natural enemies of foliage-feeding lepidopterans", whereas, predators and parasitoids that included other orders of foliage-feeding herbivores (*i.e.*, homopterans, hemipterans, and dipterans) plus non-herbivores in their diet and host range were designated "natural enemies of non-lepidopteran herbivores". Of the 546 rice-associated taxa in the cumulative Philippines-wide web, 105 taxa were recorded in this study.

### **Rice varieties and plant damage assessments**

IR64, a popularly grown, high-yielding semidwarf variety was grown at Pila and Sipit, whereas IR36, another high-yielding semidwarf variety, was planted at IRRI farm. These varieties have low to moderate resistance to stem borers (Khush, 1989) but are not resistant to leaf-folders or other foliage-feeding caterpillars (M.B.C. and A.M. Romena, personal observations). IR36 and IR64 were resistant to most populations of *N. lugens* and the green leafhopper *Nephotettix virescens* (Distant) when first released in 1975 and 1985, respectively (Khush, 1989). IR36 has retained a moderate level of resistance to *N. lugens* and *N. virescens* populations in Laguna Province, while IR64 is moderately resistant to *N. lugens* but susceptible to *N. virescens* (M.B.C., personal observations). Varietal responses of entire herbivore and natural enemy faunas are unknown for rice and most other crops (Bottrell *et al.*, 1998).

On six selected dates, plant injury caused by three lepidopteran complexes was evaluated: leaffolders (principally *Cnaphalocrocis medinalis* and *Marasmia patnalis*; Lepidoptera: Pyralidae), various other foliage-feeding caterpillar species (principally the green hairy caterpillar *Rivula nr. atimeta* (Swinhoe) and various armyworms of assorted species), and stem borers. The percentage of injured leaves or tillers was recorded on one randomly-chosen hill from each of the 10 units within each treatment for the six dates.

### **Data analysis**

Because plots within sites were unreplicated, plot means from the 10 subsamples were averaged over the three sites for each sampling date, as recommended by

Hurlbert (1984). Differences in treatments were assessed using rank-abundance plots (a), two ecostatistical measures (b–c), bioequivalence tests (d), species accumulation curves (e), and various parametric and non-parametric statistical tests (f–g). The first four methods (a–d) relied on seasonal averages or cumulative abundances of predators and parasitoids to determine treatment differences:

- (a) rank-abundance (or dominance-diversity) curves plot the abundance of taxa against decreasing rank (Bazzaz, 1975). Predator and parasitoid abundances were plotted on a  $\log_{10}$  scale with a unique number assigned to each taxon. Comparison of abundances in the *Bt*- and water-sprayed treatments was achieved using matched rank-abundance plots (Longino and Colwell, 1997) in which the water treatment was chosen as the reference plot to which the *Bt* treatment was compared. When plotted in this way, rank-abundance plots reveal biologically important aspects of species diversity (Feinsinger, 2001) and allow a quick visual check of the degree of correspondence between two treatments (Longino and Colwell, 1997);
- (b–c) two ecostatistical tests were used to analyze different aspects of community structure between treatment pairs. (b)  $E(S_n)$ , the rarefaction statistic and its variance  $\text{var } E(S_n)$  (Simberloff, 1972) were used to test the null hypothesis that water- and *Bt*-sprayed communities have the same species richness by standardizing (rarefying) total abundance of one treatment to equal the other (Gotelli and Graves, 1996; James and Rathbun, 1981). A final result such as  $E(S_{3246}) = 49 \text{ vs. } 49.8 (48.9–50.7)$  denotes species richness of the water (observed  $S$ ) and the *Bt* (expected  $S \pm 95\%$  CI) treatments, respectively, for  $n = 3246$  individuals. (c)  $r_s$ , Spearman's (1904) rank test, and its correction for excessive ties (Daniel, 1990), was used to test the null hypothesis that species-abundance rankings of sites and of water and *Bt* treatments were independent (Zar, 1984);
- (d) one-way bioequivalence tests (Dixon and Garrett, 1994; Chow and Liu, 1999) were used to test whether *Bt* sprays have no effect on natural enemy abundances at increasingly liberal thresholds. Thus, the null hypothesis was rejected if the estimated difference between treatments  $|\Delta_{\text{obs}}|$  was less than a socially-acceptable difference  $\Delta_{\text{crit}}$ , established independently of the data. Under bioequivalence testing, the burden of proof is reversed such that the treatment under study (*i.e.*, *Bt*) is considered biologically or socially important

until the evidence suggests otherwise (Steidl and Thomas, 2001).

The next three (e–g) methods were applied to time-series plots:

- (e) species accumulation (or yield-effort) curves show the rate of accumulation of species over time. Using treatment means, averaged over the three sites, accumulation curves were plotted separately for predators and parasitoids. Species accumulation curves plot the sums of the number of taxa in the previous sample and the number of taxa in the present sample that were not observed in any previous sample. For the first sample, the cumulative numbers of taxa are defined to equal its numbers of taxa;
- (f) the parametric one-tailed Student *t*-test (Zar, 1984) was used to test between-treatment differences in mean leaf injury in time-series plots and in trophic structure;
- (g) the Wald-Wolfowitz nonparametric runs test for randomness (Zar, 1984) was used to test the null hypothesis that, compared to the water-sprayed control, the pattern of departure in *Bt*-sprayed abundances for herbivores and natural enemies is random (Daniel, 1978). Departure was recorded as percentage change in abundance using the formula:  $[(\text{abundance in water-sprayed plot} - \text{abundance in } Bt\text{-sprayed plot}) / \text{abundance in water-sprayed plot}] \times 100$ . Positive numbers ( $n_1$ ) indicate that abundance in the water-sprayed plot exceeded abundance in the *Bt* plot; negative numbers ( $n_2$ ) indicate the opposite. The number of runs in the time-series,  $r$ , is the number of alternating sequences of positive or negative departures in mean abundance (zeros not included). Too many or too few runs indicate non-randomness in a time-series.

All statistical tests were judged at the nominal level of significance ( $P = 0.05$ ), and significant results in the tables are indicated with one ( $P < 0.05$ ) or two ( $P < 0.01$ ) asterisks. The 95% confidence interval (CI) for each mean, taken over the three sites, was calculated for continuous variables (leaf injuries, herbivore abundances, natural enemy abundances) in the usual way, as the treatment mean  $\pm t_{0.05, 2} \times (\text{SE, standard error of the mean})$ .

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## Appendix 1:

### Code numbers and identities of natural enemy taxa (predators, parasitoids and parasites) sampled from three rice fields in the Philippines (see Figs. 2–4).

#### Predaceous Arthropods (Adults) in *Bt*- and Water-Sprayed Plots

ID No	Taxon	Family	Order
12	<i>Anaxipha longipennis</i> (Serville)	Gryllidae	Orthoptera
13	<i>Metioche</i> sp.	Gryllidae	Orthoptera
26	<i>Orthethrum</i> sp.	Libellulidae	Odonata
31	<i>Ophionea nigrofasciata</i> (Schmidt-Goebel)	Carabidae	Coleoptera
37	<i>Menochilus sexmaculatus</i> (Fabricius)	Coccinellidae	Coleoptera
38	<i>Micraspis crocea</i> (Mulsant)	Coccinellidae	Coleoptera
46	<i>Polytoxus</i> sp.	Reduviidae	Hemiptera
83	<i>Araneus inustus</i> (L. Koch)	Araneidae	Araneae
91	<i>Oxyopes javanus</i> Thorell	Oxyopidae	Araneae
92	<i>Dyschiriognatha</i> sp.	Tetragnathidae	Araneae
93	<i>Tetragnatha mandibulata</i> Walckenaer	Tetragnathidae	Araneae
97	<i>Coleosoma blandum</i> Cambridge	Theridiidae	Araneae
119	<i>Solenopsis geminata</i> (Fabricius)	Formicidae	Hymenoptera
139	<i>Pardosa</i> sp.	Lycosidae	Araneae
141	<i>Arctosa tanakai</i> (Barrion and Litsinger)	Lycosidae	Araneae
143	<i>Oxyopes</i> spp.	Oxyopidae	Araneae
160	<i>Conocephalus</i> sp.	Tettigoniidae	Orthoptera
164	<i>Drapetis</i> sp.	Empidae	Diptera
166	<i>Ochthera</i> sp.	Ephydriidae	Diptera
167	<i>Orius tantillus</i> Motsch	Anthocoridae	Hemiptera
170	<i>Tytthus chinensis</i> Stal	Miridae	Hemiptera
171	<i>Cyrtorhinus lividipennis</i> Reuter	Miridae	Hemiptera
191	<i>Bianor</i> sp.	Salticidae	Araneae
193	<i>Clubiona</i> sp.	Clubionidae	Araneae
195	<i>Argiope aemula</i> (Walckenaer)	Araneidae	Araneae
206	<i>Thomisus</i> sp.	Thomisidae	Araneae
214	<i>Theridion</i> sp.	Theridiidae	Araneae
249	<i>Harmonia octomaculata</i> (Fabricius)	Coccinellidae	Coleoptera
250	<i>Scymnus</i> sp.	Coccinellidae	Coleoptera
251	<i>Stilbus</i> sp.	Phalacridae	Coleoptera
253	<i>Paederus</i> sp.	Staphylinidae	Coleoptera
254	<i>Nabis</i> sp.	Nabidae	Hemiptera

391	<i>Amblyseius</i> sp.	Phytoseiidae	Acari
398	<i>Atypena</i> (= <i>Callitrichia</i> ) <i>formosana</i> (Oi)	Linyphiidae	Araneae
399	<i>Marpissa</i> sp.	Salticidae	Araneae
642	<i>Anaxipha</i> sp.	Gryllidae	Orthoptera
657	<i>Campylomma</i> sp.	Miridae	Hemiptera
768	<i>Paederinae</i> sp.	Staphylinidae	Coleoptera
771	Dolichopodidae	Dolichopodidae	Diptera
778	<i>Monomorium floricola</i> (Jerdon)	Formicidae	Hymenoptera
779	<i>Paratrechina longicornis</i> (Latreille)	Formicidae	Hymenoptera
780	Coenagrionidae	Coenagrionidae	Odonata
781	Libellulidae	Libellulidae	Odonata
784	<i>Castianeira</i> sp.	Corinnidae	Araneae
791	<i>Argyodes</i> sp.	Theridiidae	Araneae
794	<i>Cunaxa</i> sp.	Cunaxidae	Acari
800	<i>Tetramorium rinatum</i> Bolton	Formicidae	Hymenoptera
803	<i>Philonthus</i> sp.	Staphylinidae	Coleoptera
806	<i>Jotus</i> sp.	Salticidae	Araneae
837	<i>Zeros</i> sp.	Staphylinidae	Coleoptera
974	<i>Parascymnus</i> sp.	Coccinellidae	Coleoptera
980	<i>Tetramorium</i> sp.	Formicidae	Hymenoptera
989	<i>Paratrechina</i> sp.	Formicidae	Hymenoptera
1016	Formicidae	Formicidae	Hymenoptera
1038	Carabidae	Carabidae	Coleoptera
1048	Staphylinidae	Staphylinidae	Coleoptera
1050	<i>Stilocopsis</i> sp.	Staphylinidae	Coleoptera
1063	Dolichopodidae	Dolichopodidae	Diptera
1064	<i>Nilobezzia</i> sp.	Ceratopogonidae	Diptera
1071	Stratiomyidae	Stratiomyidae	Diptera
1128	Ceratopogonidae	Ceratopogonidae	Diptera
1160	<i>Agriocnemis</i> sp.	Coenagrionidae	Odonata
1169	Salticidae	Salticidae	Araneae
10658	<i>Anoplolepis longipes</i> (Jerdon)	Formicidae	Hymenoptera
10691	<i>Argiope</i> sp.	Araneidae	Araneae
10701	Miridae	Miridae	Hemiptera
10746	Anthocoridae	Anthocoridae	Hemiptera
10758	Coccinellidae	Coccinellidae	Coleoptera
10772	Thomisidae	Thomisidae	Araneae
77501	Reduviidae	Reduviidae	Hemiptera
82200	Syrphidae	Syrphidae	Diptera
83812	Nabidae	Nabidae	Hemiptera
83814	<i>Tapinoma</i> sp.	Formicidae	Hymenoptera
84101	Araneidae	Araneidae	Araneae

#### Parasitoids and Parasites (Adults) in *Bt* and Water-Sprayed Plots

ID No	Taxon	Family	Order
19	<i>Scelio</i> sp.	Scelionidae	Hymenoptera
50	<i>Trichomalopsis apanteloctenae</i> Crawford	Pteromalidae	Hymenoptera

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52	<i>Telenomus rowani</i> (Gahan)	Scelionidae	Hymenoptera
57	<i>Cotesia flavipes</i> Cameron	Braconidae	Hymenoptera
66	<i>Amauromorpha accepta metathoracica</i> (Ashmead)	Ichneumonidae	Hymenoptera
67	<i>Charops brachypterum</i> Maheswary & Gupta	Ichneumonidae	Hymenoptera
72	<i>Temelucha philippinensis</i> (Ashmead)	Ichneumonidae	Hymenoptera
74	<i>Trichomma cnaphalocrosis</i> Uchida	Ichneumonidae	Hymenoptera
77	<i>Brachymeria</i> sp.	Chalcididae	Hymenoptera
78	<i>Tetrastichus</i> spp.	Eulophidae	Hymenoptera
81	<i>Eurytoma</i> sp.	Eurytomidae	Hymenoptera
109	<i>Paraphylax</i> sp.	Ichneumonidae	Hymenoptera
111	<i>Baeus</i> sp.	Scelionidae	Hymenoptera
112	<i>Idris</i> sp. 1	Scelionidae	Hymenoptera
122	<i>Megaselia scalaris</i> (Loew)	Phoridae	Diptera
124	<i>Cotesia</i> sp.	Braconidae	Hymenoptera
127	<i>Stenomesus</i> sp.	Eulophidae	Hymenoptera
129	<i>Amauromorpha</i> sp.	Ichneumonidae	Hymenoptera
131	<i>Itopectis narangae</i> (Ashmead)	Ichneumonidae	Hymenoptera
228	<i>Haplogonatopus</i> sp.	Dryinidae	Hymenoptera
231	<i>Pseudogonatopus</i> sp.	Dryinidae	Hymenoptera
242	<i>Anagrus</i> sp.	Mymaridae	Hymenoptera
243	<i>Gonatocerus</i> sp.	Mymaridae	Hymenoptera
278	<i>Opius</i> sp.	Braconidae	Hymenoptera
300	<i>Opius barrioni</i> Fischer	Braconidae	Hymenoptera
302	<i>Trichomalopsis</i> sp.	Pteromalidae	Hymenoptera
320	<i>Elasmus</i> sp.	Elasmidae	Hymenoptera
322	<i>Euplectrus</i> sp.	Eulophidae	Hymenoptera
324	<i>Cotesia angustibasis</i> (Gahan)	Braconidae	Hymenoptera
330	<i>Macrocentrus philippinensis</i> Ashmead	Braconidae	Hymenoptera
335	<i>Copidosomopsis nacoieiae</i> (Eady)	Encyrtidae	Hymenoptera
336	<i>Goniozus</i> sp.	Bethylidae	Hymenoptera
363	<i>Argyrophyllax nigrotibialis</i> (Baranov)	Tachinidae	Diptera
365	<i>Platyscelio</i> sp.	Scelionidae	Hymenoptera
380	<i>Paracentrobia</i> sp.	Trichogrammatidae	Hymenoptera
381	<i>Oligosita</i> sp.	Trichogrammatidae	Hymenoptera
383	<i>Panstenon</i> sp.	Pteromalidae	Hymenoptera
417	<i>Sepedon</i> sp.	Sciomyzidae	Diptera
423	<i>Pipunculus mutillatus</i> (Loew)	Pipunculidae	Diptera
425	<i>Tomosvaryella oryzaetora</i> (Koizumi)	Pipunculidae	Diptera
426	<i>Tomosvaryella subvirescens</i> (Loew)	Pipunculidae	Diptera
448	<i>Macroteleia</i> sp.	Scelionidae	Hymenoptera
616	<i>Gryon nixonii</i> (Masner)	Scelionidae	Hymenoptera
630	<i>Odontomyia</i> sp.	Stratiomyidae	Diptera
655	<i>Stenomesus</i> sp.	Eulophidae	Hymenoptera
738	<i>Trioxys</i> sp.	Aphidiidae	Hymenoptera
739	Agaonidae	Agaonidae	Hymenoptera
740	Alysiinae	Braconidae	Hymenoptera
744	Tetrastichinae	Eulophidae	Hymenoptera
748	<i>Telenomus</i> sp.	Scelionidae	Hymenoptera
751	<i>Psix lacunatus</i> Johnson & Masner	Scelionidae	Hymenoptera
754	Ceraphronidae	Ceraphronidae	Hymenoptera
762	<i>Idris</i> sp. 3	Scelionidae	Hymenoptera

763	<i>Mymar</i> sp.	Mymaridae	Hymenoptera
764	<i>Rogas</i> sp.	Braconidae	Hymenoptera
765	Tachinidae	Tachinidae	Diptera
844	<i>Euderus</i> sp.	Eulophidae	Hymenoptera
852	<i>Copidosomopsis</i> sp.	Encyrtidae	Hymenoptera
860	Mymaridae	Mymaridae	Hymenoptera
1019	Cynipinae	Cynipidae	Hymenoptera
1020	Diapriidae	Diapriidae	Hymenoptera
1021	<i>Trichopria</i> sp.	Diapriidae	Hymenoptera
1022	Encyrtidae	Encyrtidae	Hymenoptera
1023	Eulophidae	Eulophidae	Hymenoptera
1024	Ichneumoninae	Ichneumonidae	Hymenoptera
1026	Pteromalidae	Pteromalidae	Hymenoptera
1027	Scelionidae	Scelionidae	Hymenoptera
1158	Dacnusinginae	Braconidae	Hymenoptera
1159	Ichneumonidae	Ichneumonidae	Hymenoptera
1166	Cynipidae	Cynipidae	Hymenoptera
10632	<i>Aedes</i> sp.	Culicidae	Diptera
10650	Dryinidae	Dryinidae	Hymenoptera
83605	<i>Xorides</i> sp.	Ichneumonidae	Hymenoptera
83610	<i>Baryconus</i> sp.	Scelionidae	Hymenoptera
83809	Bethylidae	Bethylidae	Hymenoptera
84109	Braconidae	Braconidae	Hymenoptera
84110	Trichogrammatidae	Trichogrammatidae	Hymenoptera

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