

**POTENTIAL EFFECTS OF *Bt* TRANSGENE FLOW ON ARTHROPOD DIVERSITY AND ABUNDANCE IN WILD RICE, *Oryza rufipogon*, MEKONG DELTA, VIETNAM**

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**ABSTRACT**

*Many Bt endotoxins transgenic rice varieties have currently been evaluated for large-scale to release in several Asian countries within the next few years. One of the major environmental biosafety concerns regarding the release of transgenic rice is the consequence of the transgene escape of into wild and weedy rice populations. We conducted a survey in two years to document the arthropod diversity and abundance of non target Lepidoptera and their natural enemies in wild rice ecosystems in Dong Thap province, Vietnam. We found significantly greater total species diversity and greater lepidopteran diversity in wild rice (*Oryza rufipogon* Griff.) than in cultivated rice (*Oryza sativa* L.), indicating greater arthropod diversity in wild rice. Stable isotope analysis of the wild rice food web revealed that all collected arthropods were actually part of the same food web. After identifying the key Lepidopteran species associated with wild rice, we examined the susceptibility of some lepidoptera species to cry1Ac, the form Bt endotoxin transgenic rice that will be most likely released. While there was variation in susceptibility among the four lepidopteran species tested, the bioassays found that all these lepidopteran species were susceptible to the cry1Ac toxin. Therefore, Bt transgene flow has the potential to significantly impact lepidopteran-based food webs in wild rice. Data from our surveys could provide crucial information for the biosafety decisions regarding the sustainability of transgenic Bt rice in Asian countries where wild rice and relatives co-exist with the cultivated rice crops.*

**Keywords:** *Bt (Cry1Ac), Cnaphalocrosis medinalis, field survey, food web, Lepidoptera, non-target impact, Oryza rufipogon* Griff., *Oryza sativa* L.

**INTRODUCTION**

Insect-resistant transgenic crops offer significant advantages for the management of pests that are difficult to control by conventional management practices, resulting in wide adoption in many temperate countries. One of the major environmental concerns regarding the release of transgenic crops is the potential escape of transgenes into wild populations (Snow 2002, Ellstrand 2003). Wild relatives host a large assemblage of arthropods, many of which are not pests.

Therefore, transgene escape into wild plant populations can affect arthropod biodiversity, abundance, food web structure, and food web stability in wild ecosystems. While it is challenging to predict the ecological consequences of transgene flow, this proposal offers empirical evidence and a framework for determining how transgene flow may impact nontarget lepidopteran food webs.

Cultivated rice (*Oryza sativa*) naturally hybridizes only with other *Oryza* species with the AA genome. Thus in Asia, only *O. nivara*

and *O. rufipogon* would be at risk for transgene introgression. *O. rufipogon* is quite abundant and found along the margins of rice fields, marshes, and canals in the Mekong delta and in the central plains in Vietnam (Lu and Snow 2005, Cohen *et al.*, 2008). *Bt* rice may threaten wild arthropod biodiversity if the *Bt* gene enters and is fixed in *O. rufipogon* populations. If the *Bt* transgene remains intact after hybridization, the toxin could have direct lethal or sublethal effects against nontarget Lepidoptera. Lepidopteran species vary widely in their susceptibility to the *Bt* toxin (Letourneau *et al.*, 2003); susceptible specialist Lepidoptera may decline in abundance or go extinct. If this occurs, the absence of these species may affect the composition and structure of arthropod food webs and communities. Specialist natural enemies that lack of alternative hosts could experience local extirpation, while generalist natural enemies may switch to prey on other herbivores whose abundance may increase from the release from competition. There currently is very little empirical information on how *Bt* transgene flow may affect arthropod food webs in wild rice. This study will provide much needed empirical data on: 1) diversity of Lepidoptera and their natural enemies in wild rice ecosystems, 2) assess variation in nontarget lepidopteran susceptibility to *Bt* Cry toxins. By integrating empirical and theoretical approaches, we are able to characterize lepidopteran-based food webs to predict how the *Bt* gene may impact arthropod food webs in wild rice ecosystems.

## MATERIALS AND METHODS

### Lepidopteran and natural enemy species diversity and abundance in wild rice fields

Arthropod samplings were conducted in at three grasslands dominated by *O. rufipogon* located within Tram Chim National park, Phu Hiep, Phu Duc and Phu Thanh B. We assessed biodiversity in wild rice and determined determine the abundance and diversity of nontarget lepidopteran species and their natural enemies in wild rice ecosystems. As all stemborers are considered major pests, samplings were focused on nontarget

lepidopteran species. Field populations were monitored twice a month for two year, starting in January to September each year, when the flooding season arises and the wild rice *O. rufipogon* plants set seed. To reduce the possibility of sampling arthropod taxa not associated with rice, stands of wild rice were greater than 1 ha.

A combination of vacuum suction sampling, sweep-net, and hand-collection were used to sample lepidopteran and natural enemy diversity. Vacuum samples were taken using a modified leaf blower (Domingo and Schoenly 1998). A 0.4 m tall square cage was placed over a random patch of grassland. Arthropods were sucked up using a 1.5 m hose connected to a two-liter soft drink bottle attached to the bottom intake of a leaf blower. Where water depth permitted, arthropod samples were by placing the vacuum hose for 60 seconds over a patch of wild rice, and collecting them into a gauze bag. The samples were rinsed and stored in small bottles containing 70% ethanol. Ten vacuum samples were taken randomly per site on each sampling date.

In order to compare arthropod diversity and abundance between wild rice and cultivated rice, arthropod samplings were also taken at three main stages (vegetative, heading and ripening stages) of rice crops from the three cultivated fields nearby Tram Chim National Park. All arthropod samples were examined under dissecting microscopes for sorting, counting and taxonomic identification. Most of the arthropod samples were identified to species level and verified by a taxonomic consultant (A. Barrion). Using an entomological textbook, Borror and DeLong's Introduction to Study of Insects developed by Triplehorn and Johnson 2005 and a taxonomic key developed exclusively for irrigated rice (Barrion and Litsinger 1994), we were able to identify 41.7% of samples to species level. Using all available information for taxonomic identification, the remainder of the arthropod samples were identified to morpho-species and classified them into detritovore, herbivore, predator and parasitoid.

To determine the food webs of wild rice plants

*O. rufipogon*, arthropod samples were sent to UC Davis Stable Isotope, US (<http://stableisotopefacility.ucdavis.edu/>) for stable isotope analysis (SIA). Stable isotope analysis offers the potential to quickly and easily assess community structure in systems where direct observations of trophic interactions or manipulative studies are difficult (Langellotto *et al.*, 2005). Data analysis was performed by SAS Institute Inc. 2008. *JMP*® 8

### Susceptibility of lepidopteran species to CryIAc toxin

The susceptibility of four lepidopteran species collected on wild rice, (*Cnaphalocrocis medinalis*, *Mocis frugalis*, *Scirpophaga nivella* and *Scirpophaga incertulas*) against MVP II was conducted at laboratory of Entomology Department, CLRRI, in 2009, using leaf dip bioassay method. MVP II, a bioinsecticide manufactured by Mycogen Corporation, USA, containing 20% of *CryIAc*, a delta endotoxin of *Bacillus thuringiensis* var. *kurstaki* encapsulated in killed *Pseudomonas fluorescens*, this CellCap product MVP II containing *CryIAc* toxin was found to consistently enhance foliar

persistence of *Bt* endotoxin when compared to other *Bt* products.

### Leaf-dip bioassay method

Small pieces of fresh rice leaves were dipped into 0.02% Triton X-100 to remove the waxes and permit even coverage with MVP solutions, then rinsed with distilled water several times and air dried. After drying for a few minutes, they were dipped into solutions of MVP at four concentrations (0.032; 0.16; 0.8 and 4 µg/ml), then allowed to dry. The treated leaf sections were transferred to a small glass tube, plugged with non-absorbent cotton and placed on pieces of filter paper moistened with distilled water. Each cut end was covered with a small piece of moist filter paper. Five neonate larvae of test Lepidopteran species were transferred to each tube. Each treatment was replicated ten times. Larval mortality was recorded after every 24 hrs up to four days.

## RESULTS

### Arthropod diversity and abundance in wild rice *Oryza rufipogon*

**Table 1.** The composition of arthropod taxa sampled across 3 wild rice fields in 2007-2008, Dong Thap, Vietnam

No.	Order	Family (no.)	Species (no.)	Individual (no.)	Percent of total individuals (%)
1	Acari	5	6	575	1.67
2	Araneae	14	16	7235	20.98
3	Blattodea	1	2	1183	3.43
4	Coleoptera	33	41	5061	14.67
5	Dermaptera	1	1	79	0.23
6	Diptera	25	36	4757	13.79
7	Ephemeroptera	2	2	69	0.20
8	Hemiptera	34	40	7852	22.76
9	Hymenoptera	34	45	4400	12.76
10	Lepidoptera	13	17	612	1.77
11	Neuroptera	2	2	55	0.16
12	Mantodea	1	1	1	0.00
13	Odonata	2	2	137	0.40
14	Orthoptera	7	8	2444	7.09
15	Thysanoptera	3	3	22	0.06
16	Trichoptera	1	1	10	0.03
	<b>Total</b>	<b>178</b>	<b>223</b>	<b>34.492</b>	



Based on pooled data from all sample sites and dates, a total of 34,492 individual arthropods were collected by vacuum sampling over 12 sampling dates of 2 years (2007 and 2008) in wild rice fields in Tram Chim National Park of Dong Thap Province, Vietnam. A total of 223 species and morphospecies belonging to 178 families and 16 orders. Among these orders, the percentages of individuals of Hemiptera and Araneae were highest, accounted for 20.98% and 22.76%, respectively (Table 1).

Analysis of arthropod data collected in wild rice and cultivated rice revealed that arthropod abundance and diversity in wild rice was higher than in cultivated rice. Particularly, the abundance of Araneae and Hemiptera. Most of species from these orders were predators and parasitoids, while a very high number of hemipteran pest (*Nilaparvata lugens*) was found in cultivated rice (Table 2).

**Table 2.** The composition of arthropod taxa sampled in wild rice, *O. rufipogon* and cultivated rice, *O. sativa* in 2007-2008, Dong Thap, Vietnam

No.	Order	Family (no.)		Species (no.)		Individual (no.)	
		<i>O. rufipogon</i>	<i>O. sativa</i>	<i>O. rufipogon</i>	<i>O. sativa</i>	<i>O. rufipogon</i>	<i>O. sativa</i>
1	Acari	3	1	3	1	45	2
2	Araneae	12	10	27	16	1350	390
3	Blattodea	1	1	9	1	54	4
4	Coleoptera	20	8	41	8	482	50
5	Collembola	2	2	2	2	704	41
6	Dermoptera	1	1	1	1	28	2
7	Diptera	18	13	37	22	779	535
8	Ephemeroptera	1	0	1	0	1	0
9	Hemiptera	20	9	35	17	2477	4370
10	Hymenoptera	19	7	49	10	231	62
11	Lepidoptera	6	2	15	2	126	167
12	Odonata	2	0	2	0	9	0
13	Orthoptera	4	2	12	4	744	40
14	Thysanoptera	1	1	2	1	4	3
15	Trichoptera	1	0	1	0	3	0
<b>Total</b>		<b>111</b>	<b>57</b>	<b>237</b>	<b>85</b>	<b>7037</b>	<b>5666</b>

Some common arthropod diversity indices including abundance, Shannon-Weaver diversity index, and Evenness calculated for wild rice fields and cultivated rice are showed in Table 3. Remarkably, we found that the diversity index of arthropod diversity in wild rice fields were higher than in cultivated rice fields. Also, the high number of Evenness in wild rice ( $E_D = 0,2307$ ) indicated that the arthropod equitability in

wild rice was more stable than in cultivated rice ( $E_D = 0,0740$ ). The results showed that the diversity and evenness in wild rice (undisturbed habitat) are much higher than in the site from the highly disturbed habitat (cultivated rice). The wild rice habitat not only has a greater number of species present, but the individuals in the community are distributed more equitably among these species.



**Table 3.** Arthropod diversity and abundance of in wild rice and cultivated rice, Dong Thap, Vietnam

Order	Family	<i>Oryza rufipogon</i>			<i>Oryza sativa</i>		
		Individual no.	Individual proportion $p_i$	$P_i^2$	Individual no.	Individual proportion $p_i$	$P_i^2$
Acari	Oribatidae	43	0.0061	0.0000	2	0.0004	0.0000
	Others	2	0.0003	0.0000	0	0.0000	0.0000
Araneae	Lycosidae	1002	0.1424	0.0203	115	0.0203	0.0004
	Linyphiidae	92	0.0131	0.0002	163	0.0288	0.0008
	Others	257	0.0365	0.0013	112	0.0198	0.0004
Blattodea	Blattidae	54	0.0077	0.0001	4	0.0007	0.0000
Coleoptera	Carabidae	88	0.0125	0.0002	1	0.0002	0.0000
	Pselaphinae	83	0.0118	0.0001	7	0.0012	0.0000
	Coccinellidae	82	0.0117	0.0001	2	0.0004	0.0000
	Others	230	0.0327	0.0011	40	0.0071	0.0000
Collembola	Entomobryidae	679	0.0965	0.0093	38	0.0067	0.0000
	Sminthuridae	25	0.0036	0.0000	3	0.0005	0.0000
Dermaptera	Carcinophoridae	28	0.0040	0.0000	2	0.0004	0.0000
Diptera	Chironomidae	293	0.0416	0.0017	180	0.0318	0.0010
	Tipulidae	110	0.0156	0.0002	22	0.0039	0.0000
	Culicidae	105	0.0149	0.0002	54	0.0095	0.0001
	Others	270	0.0384	0.0015	279	0.0492	0.0024
Ephemeroptera	Baetidae	1	0.0001	0.0000	0	0.0000	0.0000
Hemiptera	Delphacidae	1525	0.2167	0.0470	3495	0.6168	0.3805
	Cicadellidae	464	0.0659	0.0043	59	0.0104	0.0001
	Veliidae	191	0.0271	0.0007	371	0.0655	0.0043
	Meenoplidae	150	0.0213	0.0005	1	0.0002	0.0000
	Others	146	0.0207	0.0004	444	0.0784	0.0061
Hymenoptera	Scelionidae	79	0.0112	0.0001	26	0.0046	0.0000
	Formicidae	70	0.0099	0.0001	4	0.0007	0.0000
	Braconidae	17	0.0024	0.0000	10	0.0018	0.0000
	Others	66	0.0094	0.0001	22	0.0039	0.0000
Lepidoptera	Pyralidae	102	0.0145	0.0002	166	0.0293	0.0009
	Noctuidae	11	0.0016	0.0000	0	0.0000	0.0000
	Limntriidae	6	0.0009	0.0000	0	0.0000	0.0000
	Others	6	0.0009	0.0000	1	0.0002	0.0000
Odonata	Coenagrionidae	9	0.0013	0.0000	0	0.0000	0.0000
Orthoptera	Gryllidae	651	0.0925	0.0086	37	0.0065	0.0000
	Tettigoniidae	60	0.0085	0.0001	0	0.0000	0.0000
	Others	33	0.0047	0.0000	3	0.0005	0.0000
Thysanoptera	Thripidae	4	0.0006	0.0000	3	0.0005	0.0000
Trichoptera	Psychomyiidae	3	0.0004	0.0000	0	0.0000	0.0000
Abundance		7037			5666		
Shannon's H =				2.762			1.612
Equitability ( $E_D$ ) =				0.2307			0.0740



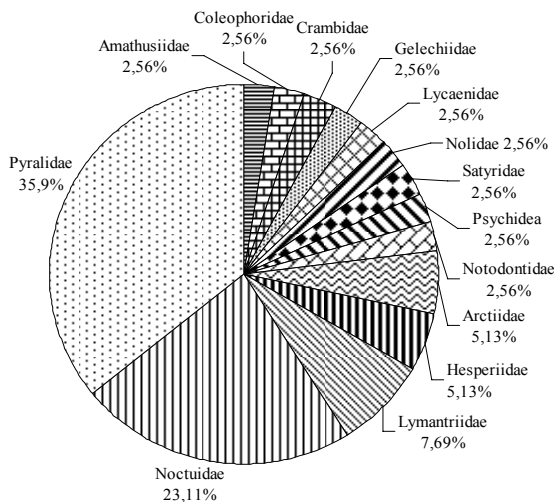


Abundance and diversity of lepidopteran species in wild rice habitat were presented by 14 families, 39 species and 758 individuals. Among these, the most abundant families were Pyralidae and Noctuidae constituted 35.9% and 23.1%, respectively (Table 4, Figure 1). The pyralid leaf folder, *Cnaphlocrocis medinalis* (Guenee) (Pyralidae) was the most dominant (54.4%), followed *Nola taeniata* (Snellen) (14,9%), *Scirpophaga nivella* (Fabricius) (6,6%). Lepidopteran diversity and abundance in wild rice habitat was much higher than in cultivated rice fields (Table 5). Obviously,

we found that wild rice hosts more number of lepidopteran species than cultivated rice. If the natural enemy is a specialist on non-target lepidopteran species that has high susceptibility to *Bt* toxin, the lepidopteran based food web are likely to decline in the wild rice ecosystems. The *Bt* toxin that is commonly used to create the transgenic *Bt* rice is widely known to affect Lepidoptera. Therefore, transgene flow could likely occur if *Bt* rice is released for commercial growth in the regions where wild rice and relatives co-exist with the cultivated rice crops.

**Table 4.** Diversity and abundance of lepidopteran species sampled in wild rice in 2007-2008 in Dong Thap, Vietnam

Family	Species no.	Individual no.	Individual proportion
Amathusiidae	1	4	0.005
Arctiidae	2	19	0.025
Coleophoridae	1	2	0.003
Crambidae	1	1	0.001
Gelechiidae	1	21	0.028
Hesperiidae	2	11	0.015
Lycaenidae	1	1	0.001
Lymantriidae	3	8	0.011
<b>Noctuidae</b>	<b>9</b>	<b>160</b>	<b>0.211</b>
Nolidae	1	2	0.003
Notodontidae	1	1	0.001
Psychidea	1	1	0.001
<b>Pyralidae</b>	<b>14</b>	<b>525</b>	<b>0.693</b>
Satyridae	1	2	0.003
<b>Total</b>	<b>39</b>	<b>758</b>	



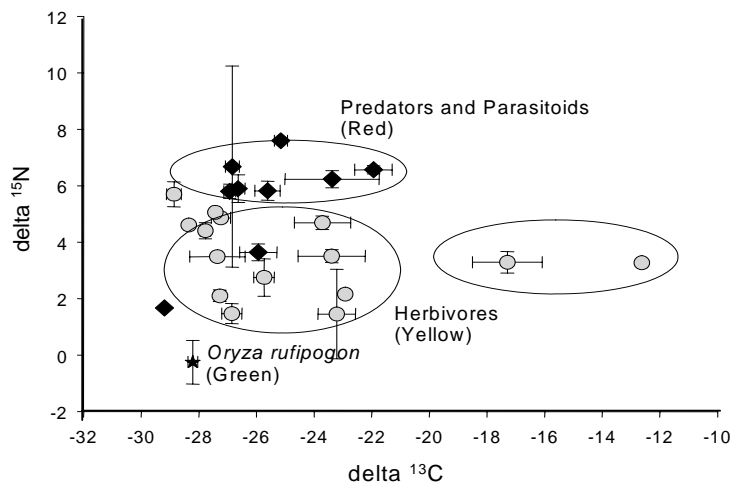
**Figure 1.** Composition of Lepidoptera families collected in wild rice habitat in 2008 and 2009

**Table 5.** Diversity indices of *Lepidopteran taxa* in wild rice and cultivated rice in Dong Thap, Vietnam

Lepidopteran Taxa	Habitat	Species diversity (S)	Shannon (H)	Evenness (E)
Family	Wild rice. <i>O. rufipogon</i>	10.00	0.98	0.43
	Cultivated rice. <i>O.sativa</i>	3.33	0.27	0.22
	<i>F</i>	*	*	*
	<i>CV (%)</i>	6.12	19.68	19.78
Species	Wild rice. <i>O. rufipogon</i>	26.00	1.85	0.57
	Cultivated rice. <i>O.sativa</i>	4.67	0.36	0.23
	<i>F</i>	*	*	*
	<i>CV (%)</i>	10.64	23.98	21.36

Data from stable isotope analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of the wild rice food web revealed that all arthropod guilds including herbivores, predators and parasitoids collected

in wild rice concentrated around wild rice plants (Figure 2). Therefore they were actually part of the same food web.

**Figure 2.** Stable isotope analysis for food web of wild rice ecosystem in Tram Chim, Dong Thap province

### Susceptibility of lepidopteran species collected in wild rice to Cry1Ac toxins

Results of leaf dip assays with four lepidopteran species. *C. medinalis*, *M. frugalis*, *S. nivella* and *S. incertulas* exposed to four different concentrations of MVP II containing Cry1Ac are presented in Table 6 – Table 9. The leaf dip bioassays showed that these lepidopteran species were susceptible to Cry1Ac (MVP II). Most of the larva survival of all Cry1Ac treatments were significantly lower than that of control at 4 days after

treated. However, there was variation in susceptibility among the four lepidopteran species tested.

Comparison of the Cry1Ac  $LC_{50}$  values indicate that *M. frugalis* was the most susceptible to Cry1Ac, followed by *S. incertulas*. While *S. nivella* was least susceptible to Cry1Ac as compared to other species. Among the lepidopteran species tested, the leaf-eating lepidopteran species such as *M. frugalis* and *C. medinalis* that were most likely to be affected by the *Bt* toxins (Table 10).

**Table 6.** Susceptibility of neonate larvae of *Cnaphalocrocis medinalis* against MVP II (Cry 1Ac). CLRRI, 2009.

Treatment	Survival (%)			
	1 DAT	2 DAT	3 DAT	4 DAT
0.032 µg/ml Cry1Ac	96.0 a	68.0 ab	28.0 ab	12.0 b
0.16 µg/ml Cry1Ac	76.0 ab	40.0 ab	16.0 bc	8.0 b
0.8 µg/ml Cry1Ac	52.0 b	16.0 b	12.0 bc	0.0 c
4 µg/ml Cry1Ac	60.0 b	12.0 b	4.0 c	0.0 c
Control	100.0 a	92.0 a	88.0 a	80.0 a
<i>F</i> <sub>cal</sub>	*	*	*	*
<i>CV</i> (%)	9.8	11.7	8.2	3.7

DAT: days after treatment

**Table 7.** Susceptibility of neonate larvae of *Mocis frugalis* against MVP II (Cry 1Ac). CLRRI, 2009.

Treatment	Survival (%)			
	1 DAT	2 DAT	3 DAT	4 DAT
0.032 µg/ml Cry1Ac	100 a	80 a	76 a	76 a
0.16 µg/ml Cry1Ac	88 ab	72 a	48 ab	44 ab
0.8 µg/ml Cry1Ac	84 b	48 b	20 bc	12 b
4 µg/ml Cry1Ac	84 b	16 c	4 c	4 b
Control	100 a	88 a	80 a	76 a
<i>F</i> <sub>cal</sub>	*	*	*	*
<i>CV</i> (%)	2.61	8.22	9.44	9.53

DAT: days after treatment

**Table 8.** Susceptibility of neonate larvae of *Scirpophaga nivella* against MVP II (Cry 1Ac). CLRRI, 2009.

Treatment	Survival (%)			
	1 DAT	2 DAT	3 DAT	4 DAT
0.032 µg/ml Cry1Ac	88.0	72.0 abc	62.0 a	42.0 a
0.16 µg/ml Cry1Ac	92.0	74.0 ab	56.0 a	19.0 b
0.8 µg/ml Cry1Ac	88.0	62.0 bc	40.0 b	10.0 bc
4 µg/ml Cry1Ac	85.0	53.0 c	29.0 b	2.0 c
Control	96.0	86.0 a	76.0 a	56.0 a
<i>F</i> <sub>cal</sub>	ns	*	*	*
<i>CV</i> (%)	2.87	7.38	9.472	14.20

DAT: days after treatment

**Table 9.** Susceptibility of neonate larvae of *Scirpophaga incertulas* against MVP II (Cry 1Ac). CLRRI, 2009.

Treatment	Survival (%)			
	1 DAT	2 DAT	3 DAT	4 DAT
0.032 µg/ml Cry1Ac	100.0 a	91.0 a	44.0 b	21.0 ab
0.16 µg/ml Cry1Ac	98.0 a	78.0 ab	42.0 b	11.0 ab
0.8 µg/ml Cry1Ac	100.0 a	70.0 ab	34.0 b	3.0 b
4 µg/ml Cry1Ac	100.0 a	61.0 b	24.0 b	3.0 b
Control	98.0	96.0 a	96.0 a	56.0 a
<i>F</i> <sub>cal</sub>	ns	*	*	*
<i>CV</i> (%)	0.6	7.9	18.8	17.1

DAT: days after treatment

**Table 10.** LC<sub>50</sub> values of four different lepidopteran species exposed to Cry1Ac (MVP II) as determined by leaf dip assays. CLRRI, 2009

Species	No. of larvae infested	Slope ± SE	LC <sub>50</sub>	95% CI	χ <sup>2</sup>
<i>C. medinalis</i>	250	1.52 ± 0.12	283.93	196.2 – 408.5	2.13
<i>M. frugalis</i>	250	0.55 ± 0.11	4.40	0.37 – 14.47	1.53
<i>S. novella</i>	250	0.61 ± 0.09	1256.74	751.3- 2521.4	0.79
<i>S. incertulas</i>	250	0.28 ± 0.08	23.62	0.39 – 87.16	0.92

## DISCUSSION

Remarkably, 70% mortality of leaf folder is due to predators (Kamal, 1981). Predators appear to be most affected by *Bt* toxins if they fed on larvae that are susceptible to *Bt* plants (Romeis *et al.*, 2006; Obrist *et al.*, 2006). Similarly, parasitoids appear to be affected if they develop within these susceptible larvae. In addition, the effects of *Bt* on non-target Lepidoptera could be mediated via altered competitive interactions or reduced control from higher trophic levels. *O. rufipogon* is found in cultivated rice fields and in uncultivated areas throughout the Mekong Delta (Watanabe *et al.*, 2000; Lu and Snow, 2005; Cohen *et al.*, 2008). Reproductive compatibility between *O. sativa* and *O. rufipogon* has been extensively demonstrated by a number of studies (Lu *et al.*, 2003; Chen *et al.*, 2004; Song *et al.*, 2004a; 2004b; Wang *et al.*, 2006). *Bt* rice may threaten wild

arthropod biodiversity if the *Bt* gene enters and is fixed in *O. rufipogon* populations. If the *Bt* transgene remains intact after hybridization the toxin can have direct lethal or sublethal effects against nontarget Lepidoptera. Lepidopteran species vary widely in their susceptibility to the *Bt* toxin (Letourneau *et al.*, 2003); susceptible specialist Lepidoptera may decline in abundance or go extinct. In general, the arthropod biodiversity in wild ecosystems is little known, especially in Lepidopteran species. Arthropod community structure in *O. rufipogon* are poorly characterized (Barrion and Litsinger, 1994). Although the diversity of Lepidoptera is high in cultivated rice fields (~250 species), but it is much lesser than lepidopteran diversity in wild rice fields. Food webs can be more reticulated in wild systems because arthropod diversity generally appears to be greater in wild systems than in cultivated systems

(Teetes and Randolph 1969. Chen and Welter 2002) and the diversity of leafhoppers and their hymenopterous parasitoids are dramatically less diverse on *Zea mays* than on *Tripsacum pilosum* and *Zea perennis*. Thus, the findings from our studies on the diversity of Lepidoptera and associated natural enemies in wild rice ecosystems in Mekong Delta are coincided with many researches so far. The results from our studies found that the arthropod biodiversity are actually higher in wild rice than in cultivated rice. Similar to previous assumptions, crop domestication may decrease arthropod diversity. Also, the intensive cultivation of rice crops can influence the abundance of several herbivorous species. In cultivated rice, we found that the arthropod community is numerically dominated by hemipteran species *Nilaparvata lugens*. On the other hand, the abundance of predators, parasitoids and detritivores are particularly high in wild rice. At least 250 species of Lepidoptera belonging to 20 families are known to feed on *O. sativa* (Barrion and Litsinger 1994. Letourneau *et al.*, 2003). Lepidopteran larvae in cultivated rice fields host a large assemblage of natural enemies: parasitoids ( $\approx 107$  species), parasites ( $\approx 5$  species), and predators ( $\approx 184$  species). Spiders and predatory beetles such as the wolf spider *Pardosa pseudoannulata* and the linyphiid spider *Atypena formosana* are the most important predators of lepidopteran larvae (Barrion, pers. comm.). For instance, Kamal (1981) attributed 70% mortality of leafhopper larvae *C. medinalis* to predators. Romeis *et al.*, 2006 found that natural enemies that feed on susceptible lepidopteran larvae experience higher mortality slower development and reduced longevity relative to controls. This suggests that some lepidopteran species may be particularly susceptible to the *Bt* endotoxin. Predators appear to be most affected by *Bt* if they are fed larvae that are susceptible to *Bt* plants (Romeis *et al.*, 2006; Obrist *et al.*, 2006). Similarly, parasitoids appear to be affected if they develop within susceptible larvae.

We found that the abundance of pyralid herbivores *C. medinalis* and *M. frugalis* are

high in wild rice. They are also highly susceptible to *Bt* toxin (*Cry1Ac*) one of the cry proteins commonly incorporated in transgenic *Bt* rice appear to have a specialized effect on Lepidopteran species. Hence, Lepidoptera species are most at risk from transgene flow of *Bt* rice into wild rice. Once *Bt* transgenes have entered wild populations the fitness of crop/wild hybrids may determine transgene persistence. Song *et al* (2004b) found that hybrids produced viable offspring but has slightly lower fitness mainly due to lower seed set and pollen viability. More recently, Xia *et al.*, (2009) demonstrated that F<sub>1</sub> hybrids between *Bt* rice and wild rice express the same levels of cry1Ac toxin content even though there are greater variability in toxin content among the *Bt* rice and wild rice hybrids. Xia *et al.*, (2009) speculated that lepidopteran insects found in wild rice may be significantly affected as the toxin levels are five times higher than the 0.01% *Bt* protein content levels shown to cause significant mortality in stem boring insects. Our results are highly relevant for discussions on the role of biodiversity in sustainable agriculture and provide important information to make predictions on how transgene flow may impact arthropod biodiversity and community structure in wild rice ecosystems. We suggest that further research should continue to explore how the *Bt* transgene flow could alter the pattern of arthropod biodiversity community structure resistance management and pest control strategy.

## CONCLUSION

The surveys on biodiversity associated with the wild rice ecosystems conducted at Tram Chim National Park, Dong Thap clearly show that this natural ecosystem contributes to sustain a rich biodiversity in wild rice, *Oryza rufipogon* G.. Our results indicate that total species diversity and evenness are greater in wild rice (*O. rufipogon*) than in cultivated rice (*O. sativa*). Also, higher lepidopteran diversity is found in wild rice than in cultivated rice. Some lepidopteran species commonly are found in wild rice, such as *M. frugalis*, *C. medinalis*, *S. novella*, and *S. incertulas*, are likely to be affected by Cry1Ac toxins. We found that many

lepidopterans are common in wild rice food webs, and they appear to be more nutritious than the delphacid and cicadellid hoppers for predators in the system. Data from stable isotope analysis for arthropods collected in wild rice indicate that all collected arthropods are actually part of the same food web. Of these, leafhopper, *C. medinalis*, appear to dominate the food webs and provide good food sources for predators to feed on. This indicates that the diversity of trophic linkages could buffer taxa at higher trophic levels from the loss of *C. medinalis* from the wild rice food web. Our results are highly relevant for discussions on the role of biodiversity in sustainable agriculture, and provide important information to make predictions on how transgene flow may impact arthropod biodiversity and community structure in wild rice ecosystems. We suggest that further research should be continued to explore how the *Bt* transgene flow could alter the pattern of arthropod biodiversity, community structure, resistance management and pest control strategy.

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**ẢNH HƯỞNG TIỀM TÀNG CỦA VIỆC TRÔI GEN *Bt* ĐẾN SỰ PHONG PHÚ VÀ ĐA DẠNG SINH HỌC CỦA CÔN TRÙNG TRÊN HỆ SINH THÁI LÚA HOANG *Oryza rufipogon* TẠI VÙNG ĐỒNG BẰNG SÔNG CỬU LONG, VIỆT NAM**

Nhiều giống lúa chuyển gen với nội độc tố *Bt* hiện đang được đánh giá nhằm phổ biến tại nhiều nước châu Á trong những năm sắp tới. Tuy nhiên có rất nhiều vấn đề về an toàn sinh học quan trọng cần phải quan tâm liên quan đến việc phóng thích những giống lúa chuyển gen *Bt*. Một trong những mối quan tâm về an toàn sinh học môi trường chủ yếu là ảnh hưởng của việc trôi gen từ cây lúa chuyển gen đến quần thể cây lúa hoang. Chúng tôi tiến hành điều tra trong thời gian 2 năm nhằm nghiên cứu sự đa dạng các loài côn trùng và mức độ phổ biến của loài côn trùng không mục tiêu thuộc bộ cánh vảy và thiên địch của chúng trên hệ sinh thái lúa hoang tại Đồng Tháp, Việt Nam. Kết quả cho thấy tính đa dạng của các loài côn trùng và loài côn trùng cánh vảy trên lúa hoang (*Oryza rufipogon* Griff.) cao hơn trên lúa trồng (*Oryza sativa* L.), chúng tỏ tính đa dạng và cấu trúc quần thể của côn trùng trên lúa hoang cao hơn. Phân tích đồng

vị ổn định của mạng lưới thức ăn của cây lúa hoang cho thấy hầu hết các loài côn trùng thu thập trên lúa hoang đều cùng mạng lưới thức ăn. Sau khi xác định những loài côn trùng cánh vảy chủ yếu trên lúa hoang, chúng tôi đánh giá mức độ mẫn cảm của một số loài côn trùng cánh vảy đối với độc tố Cry1Ac. là loại nội độc tố thường được sử dụng trong những cây lúa chuyển gen sắp được thương mại hóa. Mặc dù có sự khác nhau về mức độ nhiễm của độc tố *Bt* đối với bốn loài côn trùng cánh vảy được thử nghiệm. Các thử nghiệm sinh học cho thấy tất cả hai loài này đều mẫn cảm đối với độc tố Cry1Ac. Do vậy, việc trôi gen *Bt* có khả năng ảnh hưởng lớn đến mạng lưới côn trùng trên lúa hoang. Những số liệu điều tra và thí nghiệm trong nghiên cứu này có thể cung cấp những thông tin cần thiết nhằm hoạch định chiến lược sử dụng một cách an toàn và hiệu quả những giống lúa chuyển gen *Bt* ở những nước châu Á nơi mà lúa hoang và lúa trồng cùng hiện diện.