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## STATUS OF INSECTICIDE RESISTANCE OF THE WHITEBACKED PLANTHOPPER, *SOGATELLA FURCIFERA* (HEMIPTERA: DELPHACIDAE)

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#### Abstract

Frequent outbreaks and widespread transmission of rice black-streaked dwarf virus by Sogatella furcifera (Horváth) (Hemiptera: Delphacidae) have aggravated yield losses of rice in eastern China. The use of insecticides for suppression of the vector has been a fundamental approach to prevent epidemics of the virus disease. However, the status of insecticide resistance in S. furcifera has not been examined recently in China. In this study, dose responses of S. furcifera to buprofezin, imidacloprid, thiamethoxam, chlorpyrifos and pymetrozine were evaluated. Most populations in eastern China have developed moderate resistance to buprofezin (up to 25-fold). Approximately 32% of field populations exhibited moderate resistance to imidacloprid, while other field populations showed minor changes (7.6-fold) in their susceptibility to this insecticide. Low variation of susceptibility to thiamethoxam (<6-fold) was observed among field populations, and no obvious resistance to this product was observed. Obvious variation (10.2-fold) of susceptibility to chlorpyrifos existed in field populations of which 8% displayed moderate resistance, and 32% exhibited low level resistance. Most populations (72%) were susceptible to pymetrozine, and relatively low variation of susceptibility to it was detected among the field populations of S. furcifera. Frequent and extensive use of buprofezin had driven the rapid development of resistance, and buprofezin resistance is widespread in the field populations of S. furcifera in China. To prevent further development of the resistance, use of buprofezin should be limited and rotated with alternative insecticides with different modes of action.

Key Words: whitebacked planthopper, insecticide resistance, buprofezin, imidacloprid, thiamethoxam, chlorpyrifos, pymetrozine

## RESUMEN

Los frecuentes brotes y la transmisión generalizada del virus del enanismo de rayas negras del arroz por Sogatella furcifera (Horváth) (Hemiptera: Delphacidae) han agravado la pérdida de la producción de arroz en el este de China. El uso de insecticidas para la supresión del vector ha sido un enfoque fundamental para prevenir las epidemias de esta enfermedad viral. Sin embargo, el estado de resistencia a los insecticidas por S. furcifera recientemente no se ha examinado en China. En este estudio, se evaluaron la respuesta de S. furcifera a las dosis de buprofezin, imidacloprid, tiametoxam, clorpirifos y pimetrozina. La mayoría de las poblaciones en el este de China han desarrollado una resistencia moderada a buprofezin (hasta 25 veces). Aproximadamente el 32% de las poblaciones de campo mostraron resistencia moderada a imidacloprid, mientras que otras poblaciones de campo mostraron cambios menores (7.6 veces) en su susceptibilidad a este insecticida. Se observó una baja variación de susceptibilidad al tiametoxam (<6 veces) en las poblaciones de campo, y no se observó resistencia obvia a este producto. Si existió una obvia variación (10.2 veces) en la susceptibilidad a clorpirifos en poblaciones de campo de las cuales 8% mostraron resistencia moderada y el 32% mostraron resistencia de bajo nivel. La mayoría de las poblaciones (72%) fueron sensibles a la pimetrozina, y una variación relativamente bajo de la susceptibilidad a la que se detectó entre las poblaciones de campo de S. furcifera. El uso frecuente y extenso de buprofezin ha causado el rápido desarrollo de resistencia, y la resistencia al buprofezin está muy extendido en las poblaciones de campo de S. furcifera en China. Para evitar el desarrollo de la resistencia, el uso de buprofezin debe ser limitado y alternado con insecticidas con diferentes modos de acción.

Palabras Clave: saltahojas de espalda blanca, resistencia a los insecticidas, buprofezin, imidacloprid, tiametoxam, clorpirifos, pimetrozina The whitebacked planthopper (WBPH), *Soga*tella furcifera (Horváth) (Hemiptera: Delphacidae), and brown planthopper (BPH), *Nilaparvata* lugens Stål (Hemiptera: Delphacidae), are the most economically important insect pests on rice crops in Asian countries, and they cause serious damage by feeding and oviposition in rice stems. Both species are well known for their long-distance migratory behavior. Each year WBPH starts migration earlier than BPH. The migration of WBPH occurs mainly when rice is in the seedling and tillering stages, while that of BPH mainly occurs during the booting and heading stages. The use of insecticide sprays has been the primary method for planthopper control in most areas of China (Heong & Hardy 2009).

During the last 30 yr, the insecticides used to control rice planthopper changed greatly because of advent of new chemicals and development of insecticide resistance in target insects. At present, frequently used insecticides are buprofezin, thiamethoxam, pymetrozine, imidacloprid and chlorpyrifos. Buprofezin, an insect growth regulator (Asia et al. 1985), was introduced in China to control planthopper on rice in the late 1980s (Gao et al. 1987), and then became the main chemical for planthopper control until early 1990s. The registration of imidacloprid on rice in 1991 led to it challenging the dominant position of buprofezin, and buprofezin finally dropped out of planthopper control in China. Imidacloprid, then, played the leading role in control of rice planthopper until 2005. The development of high level resistance to imidacloprid by BPH led to its control failures in 2005 in China, which forced a change in the insecticides used for planthopper control (Wang et al. 2008a). Use of buprofezin in rice fields resurged, and chlorpyrifos, thiamethoxam and fipronil were recommended as replacements for imidacloprid. Fipronil was banned in 2009 because of high environmental risks (Nillos et al. 2009; Zhao et al. 2012) and pymetrozine then took the leading position in rice planthopper control.

Insecticide selection pressure reportedly is the major force driving the evolution of resistance in BPH (Nagata 2002; Wang et al. 2008; Liu et al. 2003). Fewer research investigations have been conducted on insecticide resistance in WBPH than on BPH (Nagata et al. 2002; Ling et al. 2009). In recent years, outbreaks of the WBPH damaged rice plants during their early stages, and the damage was aggravated by the pandemic of southern rice black-streaked dwarf virus (SRBSDV), which is transmitted by WBPH. Thus the insect and the disease caused heavy yield losses of rice throughout China, northern Vietnam, and Japan (Zhou et al. 2008; Li et al. 2012). Suppression of the WBPH vector population by frequent applications of insecticides to the earlier stages of rice (Guo et al 2010) was the only method to prevent virus disease epidemics, especially when a virus-resistant cultivar was lacking. In this study field populations of WB-PH from 9 provinces, which represent the main rice growing areas in south China, were collected and susceptibilities to frequent-used insecticides were assayed.

## MATERIALS AND METHODS

#### Insects

A laboratory reference strain (Lab-NN) of WBPH was collected from rice field in Nanning (Guangxi, China) in 2006 and reared on rice in the laboratory for 5 yr (over 50 generations) without exposure to with any insecticide. Twenty five field samples of WBPH were collected from 20 counties (or cities) in 9 provinces of China in 2010 and 2011 (Table 1, Fig. 1). Approximately 600 adults or nymphs were collected from each site, and maintained on 10-d old rice seedlings cultured in plastic boxes ( $12 \times 17 \times 38 \text{ cm}$ ) under laboratory conditions at  $27 \pm 1$  °C and 16:8 h L:D. The field-collected insects were mass mated, and the third-instar nymphs were used for bioassays.

#### Insecticides

Technical grade insecticides, except pymetrozine, were used in this study. Buprofezin (98.1%), chlorpyrifos (97%), thiamethoxam (97.2%), imidacloprid (95.8%), and pymetrozine (25%WP) were supplied by Changlong Chemical Industrial Group Co. Ltd. (Jiangsu, China), Nantong Jiangshan Agrochemical Co. Ltd. (Jiangsu, China), Syngenta Investment Co. Ltd. (Shanghai, China), Weiyuan Hebei Agrochemical Co. Ltd. (Hebei, China), and Anpon Electrochemical Co. Ltd (Jiangsu, China), respectively.

## Bioassay

The dose-responses of WBPH to different insecticides were measured using the rice-stem dipping method (Zhuang & Shen 2000). Rice plants at the tillering to booting stages were obtained and washed thoroughly. The basal 10 cm of the stems were cut and air dried to remove excess water. Three rice stems were grouped and dipped into the appropriate insecticide solution for 30 s. Three replicates were used per dose of each of 5-6 different doses of each chemical; and distilled water only was used as the control. After the rice stems had been dipped, they were air dried. The rice roots were wrapped in moistened cotton. The treated rice stems were then placed into a 500 mL plastic cup. Fifteen third-instar nymphs of S. furcifera were introduced into each plastic cup. The treated insects were maintained at  $27 \pm 1$  °C and 16:8 h L:D. Mortality was recorded after 48 h for chlorpyrifos, 96 h for thiamethoxam and imidacloprid, and 120 h for pymetrozine and buprofezin. The nymphs were considered dead if they failed to move when gently prodded with a fine bristle.

TABLE	1. LOCATIONS,	SAMPLING	DATES AND	INSECT	STAGES	OF SOGATEL	LA FURCIFER	RA COLLECTEI	) in 2010	and 2011
	FOR COMPAN	RING THE SU	USCEPTIBIL	ITIES TO	VARIOUS	INSECTICID	ES OF FIELD	POPULATIONS	OF 9 PROV	VINCES TO
	THE LAB $NN$	I REFERENC	CE STRAIN I	ESTABLIS	HED IN 2	2006 FROM A	Nanning, G	UANGXI PROV	VINCE POP	ULATION.

Map ref. no	Location	Collection date	Coordinates	Insect stage				
1	Jinping, Yunnan	Jun, 2011	N 22.77° E 103.24°	Nymph				
2	Nanning, Guangxi	Apr, 2010	N 22.84° E 108.25°	Adult				
2	Nanning, Guangxi	Jun, 2011	N 22.84° E 108.25°	Nymph, adult				
3	Cangyuan, Yunnan	Jun, 2011	N 23.15° E 99.24°	Adult				
4	Funing, Yunnan	May, 2011	N 23.62° E 105.6°	Adult				
5	Eshan, Yunnan	Jul, 2011	N 24.16° E 102.38°	Nymph				
6	Shizong, Yunnan,	Jun, 2011	N 24.85° E 103.97°	Nymph				
7	Guilin, Guangxi	Jun, 2010	N 25.28° E 110.29°	Adult				
8	Minqing, Fujian	Aug, 2011	N 26.22° E 118.86°	Nymph				
9	Gulin, Sichuan	Jul, 2010	N 28.04° E 105.82°	Nymph, adult				
9	Gulin, Sichuan	Jun, 2011	N 28.04° E 105.82°	Nymph, adult				
10	Xuyong, Sichuan	Jul, 2010	N 28.15° E 105.44°	Nymph, adult				
10	Xuyong, Sichuan	Jun, 2011	N 28.15° E 105.44°	Nymph, adult				
11	Changsha, Hunan	Aug, 2011	N 28.18° E 113.07°	Nymph				
12	Naxi, Sichuan	Aug, 2010	N 28.77° E 105.40°	Nymph, adult				
12	Naxi, Sichuan	Jul, 2011	N 28.77° E 105.40°	Nymph, adult				
13	Hejiang, Sichuan	Jun, 2011	N 28.81° E 105.82°	Nymph, adult				
14	Jiangyang, Sichuan	Jul, 2010	N 28.87° E 105.43°	Nymph, adult				
14	Jiangyang, Sichuan	Jul, 2011	N 28.87° E 105.43°	Nymph, adult				
15	Jinhua, Zhejiang	Aug, 2011	N 29.07° E 119.65°	Nymph, adult				
16	Qianshan, Anhui	Sep, 2011	N 30.63° E 116.58°	Nymph				
17	Fengxian,Shanghai	Sep, 2010	N $30.92^{\circ} \to 121.48^{\circ}$	Nymph				
18	Gaochun, Jiangsu	Aug, 2011	N 31.33° E 118.89°	Nymph, adult				
19	Yixing, Jiangsu	Aug, 2011	N 31.34° E 119.78°	Nymph				
20	Jiangpu, Jiangsu	Aug, 2010	N 32.03° E 118.87°	Adult				

<sup>a</sup>The LC<sub>50</sub> values are expressed as mg ai/L.

<sup>b</sup>Resistance factor =  $LC_{50}$  of field population/ $LC_{50}$  of Lab-NN strain.

NA: Not assayed.

## Data Analysis

Lethal concentration values (LC<sub>50</sub>) and their 95% fiducial limits (FL) were estimated using POLO-Plus program (Version 2.0) (LeOra Software 2008). A significant difference between LC<sub>50</sub> values was indicated by non-overlapping 95% fiducial limits (FL). Resistance factors (RF) were estimated at the LC<sub>50</sub> level as RF = LC<sub>50</sub> of field populations/LC<sub>50</sub> of Lab-NN population. Insecticide resistance levels were described using RFs (Lai et al. 2011) as follows: susceptibility (RF = 1), decreased susceptibility (RF = 3-5), low resistance (RF = 5-10), moderate resistance (RF = 10-40), high resistance (RF = 40-160), and very high resistance (RF > 160).

## RESULTS

Toxicities of Insecticides to the Susceptible Laboratory Reference Strain of *S. furcifera*.

The reference strain (Lab-NN) was collected at Nanning (Ref. no. 2 in Fig. 1) in 2006 and

maintained in laboratory for 5 yr (>50 generations) without contact with any insecticides. The  $LC_{50}$  values of imidacloprid, thiamethoxam, buprofezin, chlorpyrifos and pymetrozine in Lab-NN strain of *S. furcifera* were 0.109, 0.096, 0.044, 0.236 and 0.478 mg/L, respectively (Table 2). The Lab-NN strain had become 9.3-fold more susceptible to chlorpyrifos than the population from which it had been derived. The Lab-NN strain had also become slightly but not significantly more susceptible to imidacloprid, thiamethoxam and buprofezin than the original population. In this study Lab-NN was taken as the reference strain for resistance evaluation.

## Buprofezin Susceptibility of Sogatella furcifera

The toxicities of buprofezin on *S. furcifera* from different geographic areas were evaluated in 2010 and 2011 (Table 2). The LC<sub>50</sub> values of field populations varied from 0.068 mg/L in Nanning to 1.135 mg/L in Hejiang. Obvious variations of tolerance (up to 16.7-fold between the Nanning and Hejiang populations) existed among the field



Fig. 1. Sampling sites of Sogatella furcifera in rice fields of southern China in 2010 and 2011.

populations assayed. Compared with the susceptible strain 21 of 25 field populations (84%) had developed moderate resistance to buprofezin. Two populations from Minqing and Changsha showed low resistant to this chemical, i.e., 5.2-fold and 6.7-fold, respectively. The population from Nanning was the only one that was susceptible in 2010 and 2011. No significant differences of susceptibilities were observed in populations from the same locations between 2010 and 2011. These data revealed widespread resistance to buprofezin in the field populations of *S. furcifera* in China.

## Imidacloprid Susceptibility of Sogatella furcifera

A total of 25 field populations collected from 9 provinces were assayed in 2010 and 2011 for their susceptibility to imidacloprid (Table 2).  $LC_{50}$  values of field populations ranged from 0.216 in Nanning to 1.635 mg/L in Qianshan. Narrow variation of susceptibility (7.6-fold between the Nanning and Qianshan populations) existed among the

field populations from different geographic areas. Eighty-eight percent of the field populations were more tolerant than the susceptible strain. Two populations from Nanning and Naxi showed sensitivity to imidacloprid similar to the susceptible strain. Seven of 25 assayed populations (28%), from Hejiang, Guilin, Jiangpu, Yixing, Minqing, Changsha and Qianshan, showed moderate resistance to imidacloprid. Ten populations (40%) exhibited low level resistance to this insecticide. Other populations (32%) remained susceptible.

## Thiamethoxam Susceptibility of Sogatella furcifera

The toxicity (LC<sub>50</sub> values) of thiamethoxam against *S. furcifera* was in the range of 0.141 in Shizong and 0.813 mg/L in Cangyuan (Table 2), narrow variation of susceptibility (< 6-fold between the Shizong and Cangyuan populations) was observed among the 25 field populations. Compared with the LC<sub>50</sub> of laboratory reference strain (0.096 mg/L) 7 of 25 field populations (28%)

		$\mathrm{RF}^{\mathrm{b}}$	1.0		1.8	3.3	1.5	2.2	1.8	2.5	1.5	9.0			2.7	3.8	7.0	2.4	3.4	3.3	5.1	6.2	4.5	4.3	4.2	2.2	6.1	7.2	5.0	2.2
	Pymetrozine	${ m LC}_{50}~(95\%{ m FL})^{ m a}$	0.478(0.340-0.625)		0.840(0.587‐1.098)	1.589(1.005-2.335)	$0.717(0.507 { ext{-}} 0.941)$	1.049(0.690-1.433)	0.869(0.295-1.509)	$1.208(\ 0.600-1.847)$	0.706(0.419-1.009)	$4.308\left(2.022 -7.443 ight)$		NA	$1.281\left(0.446 - 3.477 ight)$	1.816(0.776 - 3.984)	$3.365\left(2.420 - 5.039 ight)$	$1.144\left(0.670\text{-}1.776\right)$	$1.613\left(1.202 \text{-} 2.163\right)$	$1.563\left(1.042 { ext{-}} 2.153 ight)$	2.425(1.477 - 4.247)	2.977(1.856 - 5.257)	$2.142\left(1.464 - 3.250 ight)$	$2.042\left(1.471 - 2.848 ight)$	$2.018\left(1.419 - 2.870 ight)$	1.034(0.547 - 1.653)	$2.963\left(2.051\text{-}4.640 ight)$	$3.426\left(2.289 - 5.614 ight)$	$2.380\left(1.683 - 3.243 ight)$	1.066(0.610-1.584)
		${f RF}^b$	1		8.2	2.5	3.2	4.1	3.2	3.0	2.2	2.7		7.2	16.3	2.9	22.9	3.0	4.1	4.8	8.3	4.4	5.6	5.0	6.1	4.4	7.8	5.4	4.9	4.1
	Chlorpyrifos	${ m LC}_{_{50}}(95\%{ m FL})^{_{8}}$	$0.236\ (0.169 - 0.312)$		1.946(1.337 - 2.695)	$0.592\ (0.356-1.086)$	$0.757\ (0.618 - 0.911)$	$0.967\ (0.720 - 1.268)$	$0.760\ (0.596-0.951)$	$0.707\ (0.578 - 0.853)$	$0.530\ (0.308-0.874)$	$0.639\ (0.480 - 0.816)$		$1.693\ (1.292 - 2.313)$	3.853(2.726 - 5.336)	$0.691\ (0.455 - 0.956)$	5.409(2.857 - 9.339)	0.711(0.481-0.976)	$0.960\ (0.692 - 1.265)$	1.122(0.769 - 1.631)	1.968(1.576-2.538)	1.045(0.785-1.344)	1.322(0.966-1.823)	1.181(0.983-1.403)	$1.438\ (1.165 - 1.766)$	$1.029\ (0.765 - 1.335)$	1.845(1.419-2.457)	$1.264\ (0.867 - 1.667)$	$1.156\ (0.677 - 1.712)$	$0.977\ (0.534 - 1.649)$
PHIC AREAS OF CHINA.		${f RF}^b$	1		3.6	5.4	4.9	3.9	4.0	4.3	5.4	6.9		2.4	8.5	3.8	2.8	1.5	2.2	3.1	3.4	3.7	2.7	3.2	3.2	4.3	5.1	8.0	2.6	5.3
	Thiamethoxam	${ m LC}_{_{50}}~(95\%{ m FL})^{_{a}}$	$0.096\ (0.040-0.169)$		$0.343\ (0.281 - 0.409)$	$0.514\ (0.359 - 0.693)$	$0.469\ (0.284 - 0.669)$	$0.372\ (0.211 - 0.548)$	$0.380\ (0.277 - 0.494)$	$0.412\ (0.317 \text{-} 0.523)$	$0.518\ (0.356 - 0.754)$	$0.660\ (0.468-0.883)$		$0.233\ (0.169 - 0.309)$	$0.813\ (0.517 \text{-} 1.757)$	$0.365\ (0.231 - 0.542)$	$0.264\ (0.190 - 0.325)$	$0.141\ (0.097 - 0.190)$	$0.216\ (0.157 - 0.287)$	0.293(0.232 - 0.373)	$0.325\ (0.173 - 0.554)$	$0.359\ (0.245 - 0.497)$	$0.259\ (0.152 - 0.419)$	$0.304\ (0.243 - 0.377)$	$0.303\ (0.120 - 0.404)$	$0.412\ (0.317 - 0.523)$	$0.491\ (0.325 - 0.848)$	$0.768\ (0.536 - 1.118)$	0.254(0.199 - 0.308)	$0.504\ (0.355 - 0.649)$
		${f RF}^b$	1		2.8	14.5	7.8	0.0	6.0	7.4	2.2	10.8		2.0	7.4	2.7	3.8	2.5	11.9	7.1	6.8	14.3	2.6	11.0	7.8	9.4	15.0	8.7	11.1	2.9
	Imidacloprid	${ m LC}_{{ m 50}}~(95\%{ m FL})^{{ m a}}$	$0.109\ (0.057 - 0.172)$		$0.300\ (0.236-0.392)$	1.585(1.018-2.606)	$0.852\ (0.606-1.107)$	$0.978\ (0.699-1.287)$	$0.649\ (0.323 - 1.073)$	$0.812\ (0.666-0.981)$	0.238(0.142 - 0.353)	$1.172\ (0.727 - 1.842)$		$0.216\ (0.160 - 0.281)$	$0.811\ (0.604 - 1.149)$	$0.298\ (0.210 - 0.392)$	$0.413\ (0.290 - 0.575)$	$0.274\ (0.287 \text{-} 0.361)$	$1.295\ (0.943 - 1.845)$	$0.776\ (0.619-1.007)$	$0.743\ (0.589 - 0.959)$	$1.558\ (0.960-2.084)$	$0.285\ (0.145 - 0.493)$	1.198(0.931-1.565)	$0.854\ (0.735 - 1.273)$	$1.020\ (0.701 - 1.510)$	1.635(1.203-2.399)	$0.943\ (0.651 - 1.530)$	$1.209\ (0.647 - 2.066)$	$0.315\ (0.185 - 0.449)$
		${f RF}^b$	1		1.5	10.7	16.1	20.2	10.1	24.9	11.9	11.6		3.1	10.1	18.8	13.3	13.4	5.2	11.3	14.5	6.7	10.4	25.6	22.5	10.8	23.9	22.2	12.3	22.3
	Buprofezin	$\mathrm{LC}_{_{50}}(95\%\mathrm{FL})^{\mathrm{a}}$	$0.044\ (0.032 - 0.059)$		$0.068\ (0.035 - 0.108)$	$0.469\ (0.269 - 0.715)$	$0.707\ (0.466 - 0.965)$	$0.887\ (0.607 - 1.222)$	$0.443\ (0.272 - 0.639)$	$1.096\ (0.667 - 1.598)$	$0.524\ (0.335 - 0.748)$	$0.511\ (0.299 - 0.773)$		$0.137\ (0.092 - 0.183)$	$0.445\ (0.314 - 0.602)$	$0.827\ (0.404 - 1.182)$	$0.583\ (0.422 - 0.774)$	$0.591 \ (0.416 - 0.796)$	$0.228\ (0.176 - 0.283)$	$0.495\ (0.383 - 0.617)$	$0.638\ (0.381 \text{-} 0.926)$	$0.293\ (0.180 - 0.414)$	$0.459\ (0.359 - 0.574)$	$1.135\ (0.895 - 1.435)$	$0.991\ (0.638-1.472)$	$0.477\ (0.318 - 0.617)$	$1.055\ (0.743 - 1.663)$	$0.978\ (0.692 \text{-} 1.490)$	$0.541\ (0.392 \text{-} 0.704)$	$0.981\ (0.572 \text{-} 1.750)$
$\mathrm{GR}^{\ell}$		Populations	Lab-NN	2010	Nanning	Guilin	Gulin	Xuyong	Naxi	Jiangyang	Fengxian	Jiangpu	2011	Nanning	Cangyuan	Funing	$\operatorname{Eshan}$	Shizong	Minqing	Gulin	Xuyong	Changsha	Naxi	Hejiang	Jiangyang	Jinhua	Qianshan	Gaochun	Yixing	Jiangpu

TABLE 2. RESPONSES TO DOSES OF INSECTICIDES BY FIELD POPULATIONS OF SOGATELLA FURCIFERA COLLECTED IN 2010 AND 2011 FROM RICE FIELDS IN DIFFERENT GEO-

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exhibited low level resistance to thiamethoxam. Other populations (72%) remained sensitive to this insecticide.

## Chlorpyrifos Susceptibility of Sogatella furcifera

All of the populations assayed were less sensitive to chlorpyrifos than the laboratory reference strain (Table 2). The LC<sub>50</sub> values of field populations ranged from 0.530 mg/L in Fengxian to 5.409 mg/L in Eshan, an obvious variation of susceptibility (10.2-fold between the Fengxian and Eshan populations) among the field populations. Two populations from Cangyuan and Eshan of Yunnan province showed moderate resistance to chlorpyrifos, while other 8 populations (32%) displayed low level of resistance. Most of the remaining populations (60%) were susceptible to chlorpyrifos.

## Pymetrozine susceptibility of Sogatella furcifera

According to the LC<sub>50</sub> values of field populations (0.706-4.308 mg/L), all of the field populations exhibited similar sensitivity to pymetrozine (Table 2). Minor variations (<5-fold between the Fengxian and Jiangpu populations) of sensitivity were observed. Results indicated that most of field populations (72%) were susceptible to pymetrozine, and only 7 populations (28%) showed low level of tolerance.

## DISCUSSION

The severe feeding damage caused by S. furcifera and the associated virus epidemic signified unsatisfactory chemical control of this planthopper. To evaluate insecticide toxicity, 2 assay methods (topical application and the rice stem dipping method) have been commonly used. Topical application assay probably could not reflect the toxicity of several insecticides. For example topical application is inappropriate to evaluate the toxicity of pymetrozine, because its mode of action is as an antifeedant (Harrewijn 1997; He et al. 2011). Thus the stem dipping method may be superior to topical application for analyzing the dose-response of pymetrozine. Similarly, the stem dipping method was suitable for any insecticides with stomach toxicity, such as buprofezin (Wang et al. 2008b). In 2009, the China Ministry of Agriculture issued an industrial standard for monitoring rice planthopper resistance to insecticide (Lin et al. 2011), and recently the stem dipping method was adopted for monitoring of planthopper resistance to insecticides (Wang et al. 2008a, 2009; Ling et al. 2009). However, baseline data of insecticide toxicity against S. furcifera have not yet been established. Only few studies reported the variation of insecticide susceptibility for field populations of *S. furcifera* in local areas, such as from Guangxi (Ling et al. 2009) and Zhejiang province (Yao et al. 2002). It was difficult to analyze the situation, especially the trend in the development of insecticide resistance by *S. furcifera* because of the absence of baseline data. After 5 years of culture in the laboratory, the strain collected from Nanning in 2006 was designated as Lab-NN. During this period the Lab-NN strain had become more susceptible to frequently used insecticides, and therefore it could be used as a susceptible reference strain for resistance monitoring (Bo et al. 2008).

Compared with reference strain Lab-NN, most of the field populations, except from Nanning, had significantly higher LC<sub>50</sub> values for buprofezin. Moderate resistance of buprofezin had developed in 84% of field populations. A similar scenario of buprofezin resistance was also presented by N. lugens (Lin et al. 2011). In 2006 and 2007 the buprofezin susceptibilities of WBPH were assayed in our laboratory, and no resistance was observed at that time; thus the resistance must have evolved in the most recent 4 yr. No resistance to buprofezin was recorded in the BPH before 2004 (Wang et al. 2008b), however in 2010 high levels of resistance to this chemical were detected in most rice growing areas of China and Vietnam (Lin et al. 2011). The flare up of resistance to buprofezin by WBPH and BPH may be caused by its increased application in rice fields as a result of the development of extremely high resistance to imidacloprid by BPH since 2005 (Gorman et al. 2008; Wang et al. 2008a).

Imidacloprid and thiamethoxam are neonicotinoid insecticides, which act as competitive inhibitors on the nicotinic acetylcholine receptors in the central nervous system. Their systemic properties and long residual activity make them ideal insecticides against sucking insects. Widespread and intensive application of imidacloprid for more than 10 yr drove the rapid development of resistance by BPH and caused its control failure in 2005. High level resistances to imidacloprid by BPH have been reported by many researchers since then (Liu et al. 2004, 2005; Liang et al. 2007; Gorman et al. 2008; Matsumura et al. 2008; Wang et al. 2008a, 2009; Matsumura & Sanada-Morimura 2010; Lin et al. 2011). The development of resistance to certain insecticides in response to their long application history may also be seen in other insects (Denholm et al. 2002; Whalon et al. 2008; Sparks et al. 2012). By contrast, WBPH resistance to imidacloprid was not obvious, and our research demonstrated that most WBPH populations from different regions remained sensitive to this chemical. The differential development of imidacloprid resistance by BPH and WBPH is an example of species specificity in the evolution of resistance. Matsumura et al (2008) revealed the species-specific development of resistance to imidacloprid and fipronil in BPH and

WBPH, respectively. The good efficacy on WBPH but poor efficacy on BPH of imidacloprid products at the present time illustrates the differential resistance development of these 2 species of rice planthopper. Although the regulatory authority partially suspended imidacloprid use against N. *lugens* in China, it can be used for WBPH control in the early stage of rice. Resistance to thiamethoxam was also not observed in field populations of WBPH, and the less than 6-fold difference of susceptibility among field populations probably reflects the variation of geographic environments, growing conditions and rice cultivation practices. A positive correlation between  $LC_{50}$  values of imidacloprid and thiamethoxam was not observed (data not shown).

Chlorpyrifos was the main insecticide for control of Asiatic rice stem borer (Chilo suppressalis [Walker]; Crambidae) and rice leaf-folder (Cnaphalocrocis medinalis [Guinée]; Crambidae) in China for yr (He et al. 2008; Zheng et al. 2011), and was also intensively used to control the rice planthopper. Fukuda & Nagata (1969) reported great differences in susceptibility to organophosphorus insecticides among geographic populations of WBPH. High levels resistance to malathion and fenitrothion were observed in Zhejiang, China during 1987-1991 (Mao & Liang 1992). These resistances remained high during 1989-1993 (Liang & Mao 1996), and declined after 1997 (Yao et al. 2000, 2002). According to our research there was 10.2-fold variation of susceptibility to chlorpyrifos among field populations of WBPH, 2 populations (Cangyuan and Eshan) displayed moderate resistance to chlorpyrifos, and most populations (60%) remained sensitive. The populations with resistance were located mainly in Yunnan and the Guangxi Autonomous Region of China, and this is where the WBPH sources for the rice growing areas of the Yangtse River Basin are located. Considerable resistance was also discovered in field populations of *C. suppressalis* in China (He et al. 2008).

Pymetrozine, a pyridine azomethine compound, is a selective insecticide against plantsucking insects, such as aphids, whiteflies, leafhoppers and planthopper (Maienfisch 2007). Pymetrozine has a novel mode of action against sucking pests in that it disrupts feeding behavior and causes insects to die of starvation (Harrewijn 1997: Foster et al. 2002: He et al. 2011). The mode of action of pymetrozine may be linked to the signaling pathway of serotonin (Kaufmann et al. 2004), which is vastly different from the modes of action of organophosphates, carbamates, neonicotinoids and other nerve poisons. However, high resistance had been detected in *Bemisia tabaci* Gennadius (Aleyrodidae) (Gorman et al. 2010; Rao et al. 2012) and Trialeurodes vaporariorum Westwood (Aleyrodidae) (Karatolos et al. 2010), and cross-resistance between neonicotinoids

and pymetrozine was found in these 2 species of whitefly. It was believed that over-expression of a cytochrome-P450 dependent monoxygenase conferred cross-resistance between neonicotinoids and pymetrozine (Gorman et al. 2010). Since 2009 pymetrozine has been the priority insecticide to control WBPH in order to prevent virus diseases vectored by it (Guo et al. 2010). Our research showed that field populations of WBPH have remained susceptible to this insecticide, and we found no obvious resistance to it. Pymetrozine resistance in BPH populations also was not discovered by our team.

The devastation caused by the virus transmitted by the WBPH drew the attention of the Agricultural Administration on WBPH control. In order to ensure the harvest of grain (rice) crop local authorities provided funding and subsidies to rice growers for the chemical control of WBPH. In 2011 the subsidized insecticides were exclusively formulations containing pymetrozine in several provinces, such as Jiangsu, Jiangxi and Anhui. Pymetrozine had been used against the rice planthopper only for a short time, and WBPH has remained susceptible to this insecticide. Nevertheless, the continued exclusive application of just one chemical along the migratory channels of WBPH in China will undoubtedly accelerate the evolution of resistance. The extremely high resistance of BPH to imidacloprid in Northeast Asia was a lesson of concerning the inevitable disastrous consequences flowing from the exclusive use over many years of imidacloprid to combat migratory BPH populations. In order to prevent or delay the development of pymetrozine resistance by the WBPH, the policy of exclusive reliance on a single type of insecticide should be replaced with a policy of using insecticides with different modes of action in a rotational use pattern. It is particularly important to avoiding the exclusive use of the same type of insecticide in emigration and immigration areas during the migratory periods of the same year. In the provinces of Guangdong, Guangxi and Yunnan, which are the sources of the WPBH for more northerly provinces of China, during May and Jun when the WBPH heavily damages the early stage of rice, pymetrozine or neonicotinoid insecticides such as imidacloprid and thiamethoxam could be used. By Jun and Jul the main areas for BWPH control have moved to the provinces of Sichuan, Chongqing, Hunan and Fujian, where chlorpyrifos could be suggested for WBPH control. Then during Aug, when Zhejiang, Jiangsu and Anhui have become the main theater of WBPH control, buprofezin and isoprocarb could be suggested. Finally in September, when the BPH has become the primary planthopper in the rice growing areas of China, the application of pymetrozine could be suggested for BPH control. The above sequence of application of different insecticides along the WBPH migration channel in a yr could disrupt the selective pressure of each insecticide on planthoppers and thereby delay or prevent the assembly of genes for resistance against each of the insecticides. Moreover, the sequence of insecticide application could be adjusted according to changes in insecticide susceptibilities of WBPH and BPH; and to make this possible, the monitoring of insecticide resistance over wider areas of rice production should be strengthened.

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