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Insecticide Resistance and Resistance Management

Monitoring Trends in Insecticide Resistance of Field Populations of *Sogatella furcifera* (Hemiptera: Delphacidae) in Guizhou Province, China, 2012–2015

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Abstract

Sogatella furcifera (Horváth) is a migratory insect that is one of the most important pest species on rice in many Asian countries. Control of *S. furcifera* (Hemiptera: Delphacidae) primarily depends on the use of chemical insecticides, and with this extensive reliance on pesticides, determining the degree of resistance of *S. furcifera* populations to the chemicals used for its control is essential. In this study, the resistance level to six conventional insecticides in five populations of *S. furcifera* from Guizhou Province was monitored yearly using the rice-stem dipping method in 2012–2015 to precisely understand current resistance levels and to estimate trends in the development of insecticide resistance in *S. furcifera* in Guizhou. Overall, *S. furcifera* from five regions in Guizhou showed a trend toward decreased susceptibility to isoprocarb (resistance ratio [RR] 0.82–3.59), susceptibility to low resistance against thiamethoxam (RR 0.27–9.69), susceptibility to moderate resistance to imidacloprid (RR 0.71–26.06), and decreased susceptibility to moderate resistance to chlorpyrifos (RR 4.63–19.58). The resistance to pymetrozine (RR 10.48–84.65) was moderate to high, and that to buprofezin (RR 6.36–412.43) was low to very high. In conclusion, the use of buprofezin and pymetrozine to control *S. furcifera* should be reduced in Guizhou Province, whereas prudent use at a reasonable frequency of chlorpyrifos and imidacloprid can continue. Isoprocarb and thiamethoxam are the best choices for effective management of *S. furcifera*. Rotations using alternative insecticides with different modes of action are recommended for regions in which resistance is at a moderate level.

Key words: *Sogatella furcifera*, neonicotinoid, pyridine, insect growth regulator, resistance monitoring

Sogatella furcifera (Horváth) (Hemiptera: Delphacidae) is one of the most serious migratory pests on rice crops throughout South and Southeast Asia (Atwal et al. 1967, Kisimoto 1971, Kisimoto 1976, Khan and Saxena 1985, Wu et al. 1997, Sogawa et al. 2009, Heong 2009, Lakshmi et al. 2010, Suri and Singh 2011, Matsumura et al. 2013). In addition to causing direct damage, *S. furcifera* is a vector of several rice pathogens, particularly the Southern rice black-streaked dwarf virus, which causes large yield losses (Shen et al. 2003, Wang et al. 2010, Pu et al. 2012, Zhou et al. 2013, Li et al. 2013, Tu et al. 2013, Lei et al. 2014).

Resistance of *S. furcifera* to the pesticides used for its control has gradually increased since the 1980s (Fukuda and Nagata 1969, Nagata and Masuda 1980, Endo et al. 1988, Endo and Tsurumachi 2001). Insecticide resistance may be one of the primary contributors to population surges, such as occurred with *Nilaparvata lugens* (Stål) (Wang et al. 2008). Before the 1990s, organophosphates, carbamates,

and pyrethroids, including dichlorvos, isoprocarb, carbaryl, deltamethrin, and cypermethrin, were used to control *S. furcifera* and *N. lugens*. From the 1970s to the 1990s, these species of rice planthopper developed a remarkable degree of resistance to the commonly used insecticides across China, Thailand, southern Vietnam, and Malaysia (Nagata and Masuda 1980, Krishnaiah and Kalode 1988, Mao and Liang 1992, Endo and Tsurumachi 2001, Nagata et al. 2002). Neonicotinoid insecticides were developed after the 1990s (Jeschke and Nauen 2008) and were used intensively against rice planthoppers, including *S. furcifera*, in many rice-growing regions (Matsumura et al. 2013). However, since 2003, these neonicotinoids have been less effective. Imidacloprid, registered for use on rice in 1991, played a key role in the management of rice planthoppers in Japan, China, and Vietnam (Liang et al. 2007, Cheng 2009). However, the high resistance to imidacloprid in *N. lugens* eventually led to control failure in China in 2005 (Wang et al. 2008, 2009a; Li et al. 2009;

Matsumura and Sanada-Morimura 2010; Matsumura et al. 2013). Buprofezin, chlorpyrifos, and thiamethoxam were recommended as replacements for imidacloprid (Bo et al. 2008). At one point, pymetrozine was the leading insecticide used for rice planthopper control (Kristinsson 1994). Currently, in China, the primary insecticides used to control *S. furcifera* include chlorpyrifos, buprofezin, pymetrozine, imidacloprid, and thiamethoxam (Zhang et al. 2014a). A number of reports suggest that *S. furcifera* has developed resistance to chlorpyrifos and buprofezin (Atwal et al. 1967, Kisimoto 1976, Nagata et al. 2002, Matsumura et al. 2013), and although the resistance of *S. furcifera* to imidacloprid and thiamethoxam remains low (Su et al. 2013, Zhang et al. 2014a), a high risk of resistance to these insecticides in the target pests remains.

Guizhou is in the center of the karst region of southwestern China, and the geographical location, environment, and climate make this province an epicenter of *S. furcifera* populations. The triangular area bounded by Guizhou, Guangxi, and Yunnan Provinces is the first stop in the migration of this pest into China. In recent years, the occurrence of *S. furcifera* in these karst regions has increased significantly (Shen et al. 2011). In 2010, *S. furcifera* attacked an accumulative area of 66,700 hectares in the Qian'nan region (southern Guizhou), with the Southern rice black-streaked dwarf virus found across 4,500 hectares (Zheng et al. 2011). In general, *S. furcifera* immigration begins in May and reaches a peak in June in Guizhou. The source of the earliest immigrants is primarily the Red River Delta of Vietnam, south-central Guangxi to vicinal Guangdong and, to a lesser extent, Hainan Island, the Leizhou Peninsula, the southwest coast of Guangdong, Laos, and central Thailand (Xue et al. 2013). This diversity of immigrant sources leads to a complex of insecticide resistance levels. Monitoring and understanding the insecticide resistance status of this pest are essential for successful resistance management in Guizhou and throughout China. However, in Guizhou, the resistance of this pest to insecticides has not been determined. Therefore, the aim of this study was to monitor the resistance dynamics of this pest to commonly used insecticides from 2012 to 2015 to provide a basis for developing strategies to manage resistance. The insecticides examined in the

study included organophosphate, carbamate, neonicotinoid, pyridine, and insect growth regulator.

Materials and Methods

Insects

Field populations of *S. furcifera* were sampled to determine resistance levels from five regions in Guizhou Province, China, during 2012–2015 (Table 1). The locations were selected according to the zoogeographical divisions of Guizhou (Li and Wang 1992). The five regions represented typical karst environments with different geomorphologies, landscapes, and vegetation in Guizhou.

Adults or nymphs were collected from paddy fields and reared for one generation on 10-d-old rice seedlings cultured in plastic boxes (34 by 23.5 by 20 cm) under laboratory conditions at room temperature. Third-instar nymphs were used for bioassays. In each of the five zoogeographical regions, adults or nymphs were collected in same paddy field for four consecutive years (2012, 2013, 2014, and 2015).

Insecticides

The following six insecticides, of technical grade, were tested: 1) the organophosphate insecticide chlorpyrifos (97.3%; Red Sun Biological Chemical Co., Ltd., Nanjing, China), 2) the carbamate insecticide isoprocarb (95%; Changlong Chemical Industrial Group Co., Ltd., Changzhou, China), 3–4) the neonicotinoid insecticides imidacloprid (97.5%; Kesheng Group Co., Ltd., Jiangsu, China) and thiamethoxam (98.3%; Syngenta Investment Co., Ltd., Shanghai, China), 5) the pyridine insecticide pymetrozine (96%; Anpon Electrochemical Co., Ltd., Jiangsu, China), and 6) the insect growth regulator insecticide buprofezin (97%; Anpon Electrochemical Co., Ltd., Jiangsu, China).

Insect Collection

To collect planthoppers, we used a colander and plastic bottles with their bottoms removed and replaced with a sponge. Cut stems of rice plants were wrapped with absorbent cotton and inserted into the bottle through the neck. In the field, the nymphs and adults were

Table 1. Locations, collection data, and insect stages of *S. furcifera* collected from 2012 to 2015 in Guizhou Province

Location	Collection date	Coordinates	Host plant	Insect stage, no.	Generation immediately before topical tests	Generation of insect at time of collection
Qianxi County, Bijie city	June, 2012	27.03° N 106.04° E	Rice	Nymph, 6,000	1	G2 or G3 in the paddy field
	July, 2013	27.03° N 106.04° E	Rice	Nymph, 7,000	1	G3 or G4 in the paddy field
	July, 2014	27.03° N 106.04° E	Rice	Nymph, 5,000	1	G3 or G4 in the paddy field
	July, 2015	27.03° N 106.04° E	Rice	Nymph, 8,000	1	G3 or G4 in the paddy field
Pingtang County, Qian'nan autonomous prefecture	June, 2012	25.83° N 107.55° E	Rice	Nymph, 4,000	1	G2 or G3 in the paddy field
	June, 2013	25.83° N 107.55° E	Rice	Nymph, 5,000	1	G2 or G3 in the paddy field
	June, 2014	25.83° N 107.55° E	Rice	Nymph, 8,000	1	G2 or G3 in the paddy field
	July, 2015	25.83° N 107.55° E	Rice	Nymph, 6,000	1	G3 or G4 in the paddy field
Bozhou District, Zunyi city	June, 2012	27.70° N 106.9° E	Rice	Nymph, 5,000	1	G2 or G3 in the paddy field
	June, 2013	27.70° N 106.9° E	Rice	Nymph, 7,000	1	G2 or G3 in the paddy field
	July, 2014	27.70° N 106.9° E	Rice	Nymph, 8,000	1	G3 or G4 in the paddy field
	July, 2015	27.70° N 106.9° E	Rice	Nymph, 5,000; Adult, 200	1	G3 or G4 in the paddy field
Pingba County, Anshun city	June, 2012	26.42° N 106.26° E	Rice	Nymph, 4,500	1	G2 or G3 in the paddy field
	June, 2013	26.42° N 106.26° E	Rice	Nymph, 8,000	1	G2 or G3 in the paddy field
	June, 2014	26.42° N 106.26° E	Rice	Nymph, 7,000	1	G2 or G3 in the paddy field
	June, 2015	26.42° N 106.26° E	Rice	Nymph, 5,000	1	G2 or G3 in the paddy field
Huaxi District, Guiyang city	June, 2012	26.40° N 106.66° E	Rice	Nymph, 5,000	1	G2 or G3 in the paddy field
	June, 2013	26.40° N 106.66° E	Rice	Nymph, 4,000	1	G2 or G3 in the paddy field
	June, 2014	26.40° N 106.66° E	Rice	Nymph, 4,000	1	G2 or G3 in the paddy field
	June, 2015	26.40° N 106.66° E	Rice	Nymph, 6,000	1	G2 or G3 in the paddy field

dislodged onto the water surface of the rice field, and then the colander was used to screen planthoppers free of the water.

Bioassay

Each insecticide was dissolved in acetone, except for pymetrozine, which was dissolved in methanol, plus 10% Triton-100 (m/V; SolarbioScience & Technology Co., Ltd., Beijing, China) as an emulsifier. The solutions of the six insecticides were then serially diluted into—five to nine different concentrations by adding distilled water.

The rice-stem dipping method (Zhuang and Shen 2000, Wang et al. 2008, Su et al. 2013, Zhang et al. 2014a) was used to test the concentration responses of *S. furcifera* to different insecticides. Rice plants at the tillering stage through the booting stage were collected and washed thoroughly. Sections (10 cm) of the basal stems had their roots cut and were air dried to remove excess water. Groups of rice stems were dipped into the prepared insecticide solutions for 30 s. Three replicates were used for each concentration, and distilled water was used as the control. After the rice stems were dipped in an insecticide, they were air dried at room temperature for at least 30 min. The roots of the rice stems were wrapped with absorbent cotton. Plastic bottles, 500 ml, with the bottoms cut off and replaced with a sponge were used as the test arena. The treated rice stems were inserted into the plastic bottles from the neck, and 20 third-instar nymphs of *S. furcifera* were introduced through the bottom of the bottles, with the sponge replaced to prevent nymph escape. Three replicates were prepared (for a total of 300–540 nymphs for each bioassay). The insects in bottles with pesticide-treated stems were maintained at $25 \pm 1^\circ\text{C}$ and a photoperiod of 16:8 (L:D) h. Mortality was recorded at 48 h for chlorpyrifos and isoprocarb, and at 96 h for imidacloprid, thiamethoxam, pymetrozine, and buprofezin. Nymphs were considered dead when they failed to move when gently prodded with a fine bristle.

Data Analyses

Mortality data were corrected for control mortality using Abbott's formula. LC_{50} values (mg liter^{-1}) and 95% fiducial limits (FLs) were calculated using DPS (Data Processing System, ver. 8.05; Hangzhou RuiFeng Information Technology Co., Ltd., Hangzhou, China; Tang and Zhang 2013). The resistance ratio (RR) was calculated by dividing the LC_{50} value of a field population by the corresponding LC_{50} value of a susceptible baseline (referred on Su et al. 2013, Zhang et al. 2014a). Insecticide resistance levels were described using the RR (Lai et al. 2011, Su et al. 2013, Zhang et al. 2014a) as follows: susceptibility ($RR < 3$), decreased susceptibility ($RR = 3-5$), low resistance ($RR = 5-10$), moderate resistance ($RR = 10-40$), high resistance ($RR = 40-160$), and very high resistance ($RR > 160$).

Results

Resistance Monitoring of *S. furcifera* to Chlorpyrifos

The resistance ratio for chlorpyrifos ranged from 4.63- to 19.58-fold higher than that of the susceptible baseline for the five populations from Guizhou sampled in four years from 2012 to 2015. All tests with chlorpyrifos revealed decreased susceptibility to a moderate resistance level in the five populations during the four years, and all RRs were significantly different from the susceptibility baseline because the 95% FLs of the RRs did not include the value 1.0. Chlorpyrifos resistance in Qianxi County (western Guizhou) and Bozhou District (Zunyi City, central Guizhou) reached 17.58 and 19.58 in 2012, respectively; decreased dramatically in 2013; and then increased in 2015. In Pingtang County (southern Guizhou) and

Huaxi District (Guiyang, central Guizhou), the resistance ratio of chlorpyrifos did not change, and no RR peak values were observed from 2012 to 2015 (RRs 12.38–16.61 and 11.48–12.82, respectively). In Pingba County (central Guizhou), the resistance ratio ranged from 4.63 to 13.54, with a peak in 2013 (13.54; Tables 2–5).

Resistance Monitoring of *S. furcifera* to Isoprocarb

The maximum resistance ratio to isoprocarb was approximately fourfold higher (RR 0.82–3.59) than that of the reference population. *S. furcifera* exhibited a tendency toward decreased susceptibility to isoprocarb but without an apparent change in the different years. The LC_{50} values of all five populations in 2015 showed no significant differences, with overlapping 95% FLs (Table 5). The isoprocarb resistance of *S. furcifera* from Qianxi ($15.37 \text{ mg liter}^{-1}$, $RR = 0.95$), Pingtang ($13.19 \text{ mg liter}^{-1}$, $RR = 0.82$), and Bozhou ($14.83 \text{ mg liter}^{-1}$, $RR = 0.92$) in 2014 and from Huaxi ($15.58 \text{ mg liter}^{-1}$, $RR = 0.97$) in 2015 was lower than the susceptibility of the baseline population ($16.13 \text{ mg liter}^{-1}$). The RRs of the Qianxi population in 2013, those of Pingba and Huaxi in 2014, and those of all five populations in 2015 were not significantly different from those of the baseline (95% FLs of RRs included the value 1.0; Tables 2–5).

Resistance Monitoring of *S. furcifera* to Pymetrozine

Pymetrozine resistance of *S. furcifera* collected from the five regions of Guizhou ranged from 6.86- to 84.65-fold higher than that of the reference population. All populations during the four years, except that in Pingba (6.86) in 2014, exhibited moderate to high resistance levels to pymetrozine (10.48–84.65), and all RRs were significantly different compared with the susceptibility baseline; the 95% FLs of RRs did not include the value 1.0. The LC_{50} values among all five populations in 2012 were not significantly different. In Pingtang, Pingba, and Huaxi, the RRs of *S. furcifera* against pymetrozine decreased from 2012 to 2014 but then increased dramatically in 2015, with RRs of 51.02, 78.37, and 84.65, respectively. The RR of the Qianxi population was at a maximum in 2014 and greatly decreased in 2015 (from 84.20 to 20.81, respectively). The RR in Bozhou District reached a maximum of 57.32 in 2012 and decreased thereafter (Tables 2–5).

Resistance Monitoring of *S. furcifera* to Buprofezin

All five field populations of *S. furcifera* in Guizhou showed very high resistance to buprofezin in 2012 (RRs from 183.18 to 1,043.18) and then slightly declined to low to very high levels of resistance (RRs from 6.36 to 412.43). All RRs, with the exception of Qianxi (6.36) in 2013, were significantly different compared with the susceptibility baseline; the 95% FLs of RRs did not include the value 1.0 (Tables 2–5).

Resistance Monitoring of *S. furcifera* to Imidacloprid

Resistance of *S. furcifera* in Guizhou to imidacloprid was at susceptible to moderate levels, with RRs significantly different compared with the susceptibility baseline; the 95% FLs of RRs did not include the value 1.0, except for the Bozhou population in 2014 (0.71). Resistance ratios of *S. furcifera* exposed to imidacloprid showed a range of increase from 0.71- to 26.06-fold compared with the reference population, except for the extreme value (63.21) of the RR in the Huaxi population in 2012. The LC_{50} value for Bozhou District ($0.08 \text{ mg liter}^{-1}$) in 2014 was lower than the susceptibility baseline ($0.11 \text{ mg liter}^{-1}$; Tables 2–5).

Table 2. Toxicity of six insecticides among five field populations of *S. furcifera* collected from different zoogeographic areas of Guizhou Province in 2012

Population (2012)	Organophosphate		Carbamate		Pyridine	
	Chlorpyrifos		Isoprocarb		Pymetrozine	
	LC ₅₀ (mg l ⁻¹) ^a / (95% FL) ^b	Slope ^c ± SE ^d RR/(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^a / (95% FL) ^b	Slope ^c ± SE ^d RR/(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^a / (95% FL) ^b	Slope ^c ± SE ^d RR/ (95% FL) ^g
Lab-NN ^e	0.24 (0.17–0.31)		16.13 (11.46–26.26)		0.48 (0.34–0.63)	
Qianxi County, Bijie city	4.15b (3.59–4.99)	3.4 ± 0.4	25.81b (22.22–29.66)	3.0 ± 0.3	22.15a (17.62–28.67)	2.2 ± 0.2
Pingfang County, Qian'nan autonomous prefecture	3.92b (3.42–4.65)	3.5 ± 0.4	32.88b (28.16–38.64)	2.6 ± 0.2	21.58a (16.06–29.43)	1.4 ± 0.2
Bozhou District, Zunyi city	4.62b (4.07–5.31)	3.5 ± 0.3	37.37c (32.33–43.87)	2.9 ± 0.3	27.40a (21.37–36.89)	2.0 ± 0.2
Pingba County, Anshun city	2.35a (2.00–2.81)	2.3 ± 0.2	31.72ab (26.21–38.60)	1.9 ± 0.2	24.45a (19.10–32.50)	2.0 ± 0.2
Huaxi District, Guiyang city	2.71a (2.24–3.37)	2.0 ± 0.2	22.71a (19.22–27.74)	2.5 ± 0.3	23.45a (19.96–28.39)	2.6 ± 0.3
Population (2012)		Insect growth regulator		Neonicotinoids		
		Buprofezin		Imidacloprid		Thiamethoxam
	LC ₅₀ (mg l ⁻¹) ^a / (95% FL) ^b	Slope ^c ± SE ^d	LC ₅₀ (mg l ⁻¹) ^a / (95% FL) ^b	Slope ^c ± SE ^d	LC ₅₀ (mg l ⁻¹) ^a / (95% FL) ^b	Slope ^c ± SE ^d RR/ (95% FL) ^g
Lab-NN ^e	0.04 (0.03–0.06)		0.11 (0.06–0.17)		0.10 (0.04–0.17)	
Qianxi County, Bijie city	35.59b (18.59–89.67)	1.6 ± 0.2	0.71a (0.56–0.92)	0.6 ± 0.1	0.51ab (0.18–1.67)	2.2 ± 0.2
Pingfang County, Qian'nan autonomous prefecture	21.36b (13.70–33.57)	2.1 ± 0.2	0.64a (0.32–1.12)	0.8 ± 0.1	1.68b (1.39–1.99)	2.0 ± 0.2
Bozhou District, Zunyi city	27.63b (22.37–36.14)	2.3 ± 0.2	0.42a (0.32–0.55)	2.0 ± 0.2	0.4a (0.34–0.47)	1.7 ± 0.2
Pingba County, Anshun city	45.9c (36.87–60.02)	2.4 ± 0.2	1.68b (1.43–1.95)	1.7 ± 0.2	1.23b (1.05–1.48)	2.7 ± 0.3
Huaxi District, Guiyang city	8.06a (6.64–9.58)	2.3 ± 0.2	6.89c (5.92–7.95)	2.1 ± 0.2	0.65b (0.53–0.78)	2.9 ± 0.3

^a LC₅₀ (mg l⁻¹): lethal concentration value expressed in mg liter⁻¹. Different letters indicate a significant difference among LC₅₀ values of the five populations based on the overlap of 95% FIs.^b 95% FL: 95% fiducial limits of LC₅₀ are in parentheses.^c Slope: the slope of the regression of LC-P line (Y=).^d SE—Standard error of slope.^e The data were referred to susceptible baselines in Su et al. (2013) and Zhang et al. (2014a).^f The resistance ratio (RR) was calculated by dividing the LC₅₀ value of a field population by the corresponding LC₅₀ value of the susceptible baseline (Lab-NN).^g 95% FL: 95% fiducial limits of resistance ratio are in parentheses. Resistance ratio is significant (fiducial limits do not include the value 1.0).

Table 3. Toxicity of six insecticides among five field populations of *S. furcifera* collected from different zoogeographic areas of Guizhou Province in 2013

Population (2013)	Organophosphate			Carbamate			Pyridine		
	Chlorpyrifos			Isoprocarb			Pymetrozine		
	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR ^f /(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR ^f /(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR ^f /(95% FL) ^g
Lab-NN ^e	0.24 (0.17–0.31)			16.13 (11.46–26.26)			0.48 (0.34–0.63)		
Qianxi County, Bijie city	1.28a (0.99–1.56)	3.6 ± 0.5	5.42 (4.13–6.50)	17.11a (14.23–20.98)	3.5 ± 0.5	1.06 (0.88–1.30)	5.01a (2.57–8.14)	1.2 ± 0.2	10.48 (5.35–16.96)
Pingba County, Qian'nan autonomous prefecture	3.89 b (2.83–6.30)	1.8 ± 0.3	16.48 (11.79–26.25)	57.96b (42.77–91.58)	1.9 ± 0.3	3.59 (2.65–5.68)	23.60b (14.97–45.79)	1.4 ± 0.2	49.37 (31.19–95.40)
Bozhou District, Zunyi city	1.15a (0.78–1.50)	2.1 ± 0.3	4.86 (3.25–6.25)	21.47a (17.61–27.66)	3.2 ± 0.4	1.33 (1.09–1.71)	18.83b (12.96–31.03)	1.8 ± 0.3	39.39 (27.00–64.65)
Pingba County, Anshun city	3.19b (2.65–3.93)	2.1 ± 0.3	13.54 (11.04–16.38)	19.07a (16.76–22.08)	3.5 ± 0.3	1.18 (1.04–1.37)	5.36a (3.91–7.40)	1.3 ± 0.1	11.22 (8.15–15.42)
Huaxi District, Guiyang city	3.02b (2.61–3.62)	3.1 ± 0.3	12.82 (10.88–15.08)	25.23a (21.21–31.34)	2.6 ± 0.3	1.56 (1.31–1.94)	19.29b (15.92–23.32)	2.0 ± 0.2	40.37 (33.17–48.58)
Population (2013)	Insect growth regulator			Neonicotinoids					
	Buprofezin			Imidacloprid			Thiamethoxam		
	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR ^f /(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR ^f /(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR ^f /(95% FL) ^g
Lab-NN ^e	0.044 (0.03–0.06)			0.11 (0.06–0.17)			0.10 (0.04–0.17)		
Qianxi County, Bijie city	0.28a (0.04–0.71)	0.9 ± 0.2	6.36 (0.91–16.14)	0.65b (0.43–0.89)	2.2 ± 0.3	5.96 (3.91–8.09)	0.33b (0.21–0.46)	1.9 ± 0.3	3.44 (2.19–4.79)
Pingba County, Qian'nan autonomous prefecture	1.31b (0.73–2.49)	0.9 ± 0.1	29.77 (16.59–56.59)	2.77c (2.03–4.27)	1.7 ± 0.2	25.41 (18.45–38.81)	0.93c (0.7–1.29)	1.67 ± 0.2	9.69 (7.29–13.44)
Bozhou District, Zunyi city	0.49a (0.22–0.89)	0.9 ± 0.1	11.14 (5.00–20.23)	2.84c (1.82–5.65)	1.7 ± 0.3	26.06 (16.55–51.36)	0.38b (0.26–0.54)	2.1 ± 0.3	3.96 (2.71–5.63)
Pingba County, Anshun city	4.37c (2.93–6.56)	0.9 ± 0.1	99.28 (66.59–149.09)	0.20a (0.14–0.27)	1.6 ± 0.2	1.86 (1.27–2.45)	0.15a (0.09–0.20)	1.6 ± 0.2	1.52 (0.94–2.08)
Huaxi District, Guiyang city	0.44a (0.30–0.60)	1.4 ± 0.1	9.90 (6.82–13.64)	1.08b (0.77–1.60)	1.2 ± 0.1	9.90 (7.00–14.55)	0.13a (0.09–0.17)	1.5 ± 0.2	1.31 (0.94–1.77)

^a LC₅₀ (mg l⁻¹); lethal concentration value expressed in mg liter⁻¹. Different letters indicate a significant difference among LC₅₀ values among the five populations based on the overlap of 95% FLs.^b 95% FL: 95% fiducial limits of LC₅₀ are in parentheses.^c Slope: the slope of the regression of LC-P line (Y=).^d SE —Standard error of slope.^e The data were referred to susceptible baselines in Su et al. (2013) and Zhang et al. (2014a).^f The resistance ratio (RR) was calculated by dividing the LC₅₀ value of a field population by the corresponding LC₅₀ value of the susceptible baseline (Lab-NN).^g 95% FL: 95% fiducial limits of resistance ratio are in parentheses. Resistance ratio is significant (fiducial limits do not include the value 1.0).

Table 4. Toxicity of six insecticides among five field populations of *S. furcifera* collected from different zoogeographic areas of Guizhou Province in 2014

Population (2014)	Organophosphate			Carbamate			Pyridine		
	Chlorpyrifos			Isoprocarb			Pymetrozine		
	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g
Lab-NN ^e	0.24 (0.17–0.31)			16.13 (11.46–26.26)			0.48 (0.34–0.63)		
Qianxi County, Bijie city	2.85b (2.26–3.80)	2.4 ± 0.4	12.06 (9.42–15.83)	15.37a (12.81–18.49)	3.6 ± 0.5	0.95 (0.79–1.15)	40.25b (28.28–65.94)	2.1 ± 0.3	84.20 (58.92–137.38)
Pingtang County, Qian'nan autonomous prefecture	2.92b (2.52–3.51)	2.9 ± 0.2	12.38 (10.50–14.63)	13.19a (11.15–15.46)	1.9 ± 0.3	0.82 (0.69–0.96)	5.67a (4.21–7.74)	1.9 ± 0.2	11.87 (8.77–16.13)
Bozhou District, Zunyi city	1.21a (0.96–1.56)	2.4 ± 0.3	5.12 (4.00–6.50)	14.83ab (11.98–20.24)	2.7 ± 0.5	0.92 (0.74–1.25)	6.61a (3.19–16.02)	0.8 ± 0.2	13.83 (6.65–33.38)
Pingba County, Anshun city	1.09a (0.92–1.27)	2.5 ± 0.2	4.63 (3.83–5.29)	31.89c (24.66–45.63)	1.6 ± 0.2	1.98 (1.53–2.83)	3.28a (2.45–4.48)	1.4 ± 0.1	6.86 (5.10–9.33)
Huaxi District, Guiyang city	2.77b (2.06–4.08)	2.2 ± 0.4	11.74 (8.58–17.00)	20.62bc (16.44–25.73)	2.8 ± 0.5	1.28 (1.02–1.60)	4.93a (3.39–7.15)	1.8 ± 0.3	10.32 (7.06–14.90)
Neonicotinoids									
Population (2014)	Insect growth regulator			Imidacloprid			Thiamethoxam		
	Buprofezin								
Lab-NN ^e	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{a/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g
Qianxi County, Bijie city	0.04 (0.03–0.06)			0.11 (0.06–0.17)			0.10 (0.04–0.17)		
Pingtang County, Qian'nan autonomous prefecture	7.12ab (3.58–17.32)	0.7 ± 0.1	161.81 (81.36–393.64)	1.15c (0.71–2.17)	1.2 ± 0.2	10.57 (6.45–19.73)	0.25b (0.17–0.38)	1.6 ± 0.2	2.61 (1.77–3.96)
Bozhou District, Zunyi city	18.15b (12.90–26.05)	1.0 ± 0.1	412.43 (293.18–592.05)	1.31c (1.10–1.59)	2.2 ± 0.2	12.03 (10.00–14.45)	0.51c (0.40–0.62)	1.9 ± 0.2	5.27 (4.17–6.46)
Pingba County, Anshun city	4.14a (2.61–6.68)	1.2 ± 0.2	94.08 (59.32–151.82)	0.08a (0.03–0.14)	1.1 ± 0.2	0.71 (0.27–1.27)	0.06a (0.04–0.09)	1.6 ± 0.2	0.64 (0.42–0.94)
Huaxi District, Guiyang city	7.13a (5.15–10.38)	1.2 ± 0.1	162.13 (117.05–235.91)	0.44b (0.31–0.62)	1.1 ± 0.1	4.02 (2.82–5.64)	0.46bc (0.33–0.65)	1.2 ± 0.1	4.84 (3.44–6.77)
	6.78a (4.00–12.90)	1.0 ± 0.2	154.00 (90.91–293.18)	0.66bc (0.41–1.11)	1.2 ± 0.2	6.08 (3.73–10.09)	0.28b (0.20–0.39)	2.1 ± 0.3	2.87 (2.08–4.06)

^a LC₅₀ (mg l⁻¹): lethal concentration value expressed in mg liter⁻¹. Different letters indicate a significant difference among the five populations based on the overlap of 95% FLs.^b 95% FL: 95% fiducial limits of LC₅₀ are in parentheses.^c Slope: the slope of the regression of LC-P line (Y=).^d SE—Standard error of slope.^e The data were referred to susceptible baselines in Su et al. (2013) and Zhang et al. (2014a).^f The resistance ratio (RR) was calculated by dividing the LC₅₀ value of a field population by the corresponding LC₅₀ value of the susceptible baseline (Lab-NN).^g 95% FL: 95% fiducial limits of resistance ratio are in parentheses. Resistance ratio is significant (fiducial limits do not include the value 1.0).

Table 5. Toxicity of six insecticides among five field populations of *S. furcifera* collected from different zoogeographic areas of Guizhou Province in 2015

Population (2015)	Organophosphate			Carbamate			Pyridine		
	Chlorpyrifos			Isoprocarb			Pymetrozine		
	LC ₅₀ (mg l ⁻¹) ^{d/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{d/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{d/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g
Lab-NN ^e	0.24 (0.17–0.31)			16.13 (11.46–26.26)			0.48 (0.34–0.63)		
Qianxi County, Bijie city	2.17ab (1.72–2.71)	2.8 ± 0.4	9.19 (7.17–11.29)	19.72a (15.70–25.88)	2.4 ± 0.3	1.22 (0.97–1.60)	9.95a (6.71–14.73)	1.6 ± 0.2	20.81 (13.98–30.69)
Pingtang County, Qian'nan autonomous prefecture	3.76b (3.04–4.97)	2.0 ± 0.2	15.93 (12.67–20.71)	18.57a (14.45–24.78)	2.1 ± 0.3	1.15 (0.90–1.54)	24.39ab (12.28–92.94)	1.0 ± 0.2	51.02 (25.58–193.65)
Bozhou District, Zunyi city	2.50b (2.02–3.20)	2.7 ± 0.4	10.58 (8.42–13.33)	16.32a (12.73–21.22)	2.2 ± 0.3	1.01 (0.79–1.32)	10.82a (7.34–16.12)	1.6 ± 0.2	22.63 (15.29–33.58)
Pingba County, Anshun city	1.52a (1.17–2.0)	1.9 ± 0.2	6.45 (4.88–8.33)	16.95a (14.05–20.81)	3.4 ± 0.4	1.05 (0.87–1.29)	37.46b (24.93–55.76)	1.5 ± 0.2	78.37 (51.94–116.17)
Huaxi District, Guiyang city	3.01b (2.32–4.2)	2.1 ± 0.3	12.77 (9.67–17.50)	15.58a (12.72–19.17)	3.0 ± 0.4	0.97 (0.79–1.19)	40.46b (27.83–58.93)	1.7 ± 0.2	84.65 (57.98–122.77)
Population (2015)	Insect growth regulator			Neonicotinoids					
	Buprofezin			Imidacloprid			Thiamethoxam		
	LC ₅₀ (mg l ⁻¹) ^{d/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{d/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g	LC ₅₀ (mg l ⁻¹) ^{d/} (95% FL) ^b	Slope ^c ± SE ^d	RR/(95% FL) ^g
Lab-NN ^e	0.04 (0.03–0.06)			0.11 (0.06–0.17)			0.10 (0.04–0.17)		
Qianxi County, Bijie city	0.50a (0.26–0.85)	1.0 ± 0.1	11.38 (5.91–19.32)	0.36a (0.25–0.49)	1.8 ± 0.2	3.30 (2.29–4.50)	0.23b (0.12–0.39)	1.5 ± 0.2	2.44 (1.25–4.06)
Pingtang County, Qian'nan autonomous prefecture	0.75a (0.42–1.30)	1.0 ± 0.1	17.11 (9.55–29.55)	1.42b (0.85–2.88)	1.1 ± 0.2	12.98 (7.80–26.42)	0.53c (0.34–0.84)	1.3 ± 0.2	5.54 (3.54–8.75)
Bozhou District, Zunyi city	2.37b (1.42–4.09)	1.0 ± 0.1	53.91 (32.27–92.95)	0.51a (0.33–0.80)	1.3 ± 0.2	4.69 (3.03–7.34)	0.09b (0.06–0.14)	1.7 ± 0.2	0.98 (0.63–1.46)
Pingba County, Anshun city	5.44c (3.63–8.61)	1.4 ± 0.2	123.59 (82.50–195.68)	0.22a (0.12–0.34)	1.3 ± 0.2	1.99 (1.10–3.12)	0.03a (0.02–0.04)	1.8 ± 0.2	0.27 (0.21–0.42)
Huaxi District, Guiyang city	1.10ab (0.68–1.84)	1.0 ± 0.2	25.05 (15.45–41.82)	0.46a (0.30–0.70)	1.4 ± 0.2	4.19 (2.75–6.42)	0.23b (0.13–0.34)	1.3 ± 0.2	2.37 (1.35–3.54)

^a LC₅₀ (mg l⁻¹): lethal concentration value expressed in mg liter⁻¹. Different letters indicate a significant difference among LC₅₀ values among the five populations based on the overlap of 95% FLs.^b 95% FL: 95% fiducial limits of LC₅₀ are in parentheses.^c Slope: the slope of the regression of LC-P line (Y =).^d SE—Standard error of slope.^e The data were referred to susceptible baselines in Su et al. (2013) and Zhang et al. (2014a).^f The resistance ratio (RR) was calculated by dividing the LC₅₀ value of a field population by the corresponding LC₅₀ value of the susceptible baseline (Lab-NN).^g 95% FL: 95% fiducial limits of resistance ratio are in parentheses. Resistance ratio is significant (fiducial limits do not include the value 1.0).

Resistance Monitoring of *S. furcifera* to Thiamethoxam

The LC_{50} value of thiamethoxam against *S. furcifera* populations collected from the five different regions in Guizhou Province tended to decrease from 2012 to 2015. The resistance ratio of *S. furcifera* to thiamethoxam indicated a trend toward low resistance level ($RR=0.27-9.69$), except for the two extreme value in the Pingtang population ($RR=17.50$) and Pingba population ($RR=12.81$) in 2012. LC_{50} values from Bozhou District ($0.06 \text{ mg liter}^{-1}$) in 2014 and Bozhou District ($0.09 \text{ mg liter}^{-1}$) and Pingba County ($0.03 \text{ mg liter}^{-1}$) in 2015 were all lower than the susceptibility baseline ($LC_{50}=0.10 \text{ mg liter}^{-1}$). The RRs of all five populations in 2012; those from Qianxi, Pingtang, and Bozhou in 2013; the four populations except Bozhou in 2014; and those from Qianxi, Pingtang, and Huaxi Counties in 2015 were significantly different from the susceptibility baseline; the 95% FLs of RRs did not include the value 1.0 (Tables 2–5).

Discussion

As the use of chemical insecticides has increased in importance for the control of rice hoppers, resistance to a number of insecticides has been reported in various planthopper species (He et al. 2013, Zhang et al. 2014a), ultimately leading to control failure. For example, during an outbreak of *N. lugens* in China in 2005, imidacloprid was suspended for control of this pest insect because of high resistance (Wang et al. 2008). To prevent such resistance-related control failure and to maintain the long-term efficacy of insecticides, the susceptibility levels of different planthopper species to the insecticides used for their control must be periodically assessed (Zhang et al. 2014a).

A common insecticide used for controlling planthoppers in rice is chlorpyrifos, a broad-spectrum organophosphate (Fukuda and Nagata, 1969) that is used intensively in China. As shown by Su et al. (2013), such intensive use resulted in a 10.2-fold difference in susceptibility to chlorpyrifos among *S. furcifera* populations from sites in Cangyuan and Eshan, China, whereas almost 60% of populations in central China displayed moderate resistance but remained sensitive to chlorpyrifos in 2011. A recent study further found that *S. furcifera* resistance to chlorpyrifos is ubiquitous in rice-planting areas of China, with resistance levels ranging from low to high and resistance ratios varying from 9.2- to 127.6-fold higher than those of the reference population in 2013 (Zhang et al. 2014a). We found that the resistance ratios for this compound ranged from 4.63 to 19.58; therefore, the resistance ranged from decreased susceptibility to moderate resistance among the five Guizhou populations from 2012 to 2015. However, in some populations, such as those from Pingtang, Bozhou, and Huaxi, RRs were 15.93, 10.58, and 12.77, respectively, suggesting a risk of further increase in resistance to chlorpyrifos.

Isoprocarb is a carbamate insecticide that has not only a knock-down effect but also a long residual effect against planthoppers (Endo et al. 1988); however, the intrinsic level of absorption is relatively low compared with the other tested compounds. Notably, all assayed populations remained susceptible to this compound. Our results showed that *S. furcifera* remained either susceptible or with decreased susceptibility to isoprocarb from all five regions in Guizhou during 2012–2015, which might be attributed to its less common use in these regions.

Buprofezin is an insect growth regulator with a long history of use for planthopper control in most rice-growing areas of China and Vietnam. *Sogatella furcifera* assayed in 2006 and 2007 showed no apparent resistance to buprofezin (Su et al. 2013, Zhang et al. 2014a). However, greater use of buprofezin may lead to a rapid increase in the resistance of *S. furcifera* because the development of

high resistance to imidacloprid in *N. lugens* occurred beginning in 2005 (Gorman et al. 2008, Ling et al. 2011). Zhang et al. (2014a) showed that many field populations of *S. furcifera* have developed dramatically high resistance to buprofezin, such that the RR increased from 10.8 in 2011, to 23.9 in 2012 and to 90.6 in 2013 in the populations collected from Jinhua City, Zhejiang Province. The LC_{50} value of buprofezin in *S. furcifera* ranged from $0.068 \text{ mg liter}^{-1}$ in Nanning, Guangxi Province, to $1.135 \text{ mg liter}^{-1}$ in Hejiang, Sichuan Province, in 2010 and 2011 (Su et al. 2013). A similar increase in buprofezin resistance was also observed in *N. lugens* in China and Vietnam (Ling et al. 2011). Compared with the reference strain Lab-NN (Su et al. 2013), our test found significantly higher LC_{50} values for this chemical, and resistance to this chemical was at least at moderate levels in most Guizhou populations. Similarly, in 2012, the maximum RR appeared in the Pingba population (1043.18), and the RR values fluctuated from 183.18 to 1043.18. According to our investigation in 2012, buprofezin was used at a lower frequency, and farmer spraying was irrational in Guizhou. Farmers, on their own initiative, increased the dosage of buprofezin application because they desired excellent control of adult *S. furcifera*; however, the farmers were unaware that buprofezin is effective only against nymphs. In 2015, resistance to buprofezin was observed in Bozhou (53.91) and Pingba (123.59). These results indicated that buprofezin has been overused, and therefore, alternative strategies, such as decreasing or even restricting buprofezin use and rotations with other insecticides, should be employed to slow the development of resistance.

Imidacloprid and thiamethoxam are neonicotinoid insecticides used for planthopper control that act as competitive inhibitors to the nicotinic acetylcholine receptors in the central nervous system. Their systemic properties and long residual activity make them ideal insecticides against sucking insects such as *N. lugens* and *S. furcifera*. However, the widespread and intensive use of imidacloprid for more than two decades since 1992 has caused a remarkable increase in resistance in *N. lugens*, leading to control failure of this pest in 2005 (Wu et al. 1997; Wang et al. 2008, 2009a, 2009b). Resistance in *S. furcifera* to imidacloprid was reported previously at a low level ($RR=0.8-12$; Su et al. 2013), and we demonstrated that most *S. furcifera* populations from the five regions in Guizhou remained relatively sensitive to this chemical ($RR=0.71-26.06$), except for the Huaxi population in 2012 ($RR=63.21$). The findings of the present study differed from those of Su et al. (2013) and Zhang et al. (2014a). However, in the Huaxi population in 2012, a high LC_{50} value was recorded, which might be attributed to the long and improper use of this compound in this region. Since 2005, the frequency of imidacloprid use has been generally low in China, which might explain the slowing increase in imidacloprid resistance observed in recent years. Similarly, no obvious resistance in *S. furcifera* to thiamethoxam was found in a recent study, with little variation in susceptibility among 25 field populations collected from nine provinces of southern China in 2011. Compared with the LC_{50} values of the laboratory reference strain ($0.10 \text{ mg liter}^{-1}$), 28% of field populations exhibited a low level of resistance to thiamethoxam and 72% remained sensitive to this insecticide (Su et al. 2013). In another study, the resistance levels of 15 field populations of *S. furcifera* to thiamethoxam were minimal and ranged from susceptible (no-resistance) to decreased susceptibility in populations from seven provinces of southern China in 2012 and 2013 (Zhang et al. 2014a). Our results also showed that the resistance of *S. furcifera* to thiamethoxam was at susceptible to low levels ($RR=0.27-9.69$) except for that of Pingtang ($RR=17.50$) and Pingba ($RR=12.81$) populations in 2012. However, the development of

thiamethoxam resistance should be a serious concern because thiamethoxam not only is one of the primary insecticides used to control rice planthoppers, including *S. furcifera*, but also has the same mode of action as imidacloprid (Zhang et al. 2014b).

Pymetrozine, a novel-activity pyridine-azomethine, is a selective insecticide effective against plant-sucking insects such as aphids, whiteflies, leafhoppers, and planthoppers, yet relatively safe to their natural enemies (Kristinsson 1994). Additionally, the mode of action of pymetrozine is completely different from that of organophosphates, carbamates, neonicotinoids, and other nerve poisons. However, high resistance to pymetrozine has been detected in *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in China, the United States, and Spain, among other countries (Gorman et al. 2010, Rao et al. 2012), and in *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in China (Karatosol et al. 2010). According to Su et al. (2013), several field populations of *S. furcifera* exhibited similar sensitivity to pymetrozine (0.706–4.308 mg liter⁻¹). Minor variations (less than fivefold) were observed in sensitivity between Fengxian (in Shanghai City) and Jiangpu (in Nanjing City, Jiangsu Province) populations, and 72% of field populations were susceptible to pymetrozine, with 28% exhibiting a low tolerance. Our results showed that pymetrozine resistance in *S. furcifera* in the five regions of Guizhou ranged from 10.48 to 84.65, representing a medium-to-high resistance level, except in the Pingba population (6.86) in 2014. In some regions of Guizhou, such as Pingtang (51.02), Pingba (78.37), and Huaxi (84.65) in 2015, resistance to pymetrozine developed to a high level. Considering that pymetrozine has been widely used throughout China, monitoring the development of pymetrozine resistance in Guizhou and other regions is critical.

In the five populations during the four years, we found the minimum LC₅₀ values for isoprocarb, imidacloprid, and thiamethoxam, i.e., 13.19, 0.08, and 0.03 mg liter⁻¹, respectively, which were all lower than the susceptibility baselines of 16.13, 0.11, and 0.10 mg liter⁻¹, respectively. However, these lower LC₅₀ values should not be regarded as the new susceptibility baselines for these three chemicals in Guizhou because the adoption of a new susceptibility baseline depends not only on the LC₅₀ value but also on other factors, such as different operators and conditions, including rice variety, stage or health, and room condition, among others.

To determine the most effective strategies for control of *S. furcifera* in the future, constant monitoring of insecticide resistance is recommended for Guizhou. According to this study, chlorpyrifos, imidacloprid, thiamethoxam, and isoprocarb are suitable choices for pest control in a program of rotating insecticides for resistance management. From year-to-year, the level of resistance to different insecticides among different regions of Guizhou fluctuated dramatically. Differences in insecticide application might be a key factor in these fluctuations in resistance. Moreover, *S. furcifera* is a long-distance migratory pest, and therefore, insecticide application and consequent evolution of resistance in one region could theoretically influence the development of resistance in another region (Zhuang and Shen 2000). Thus, resistance monitoring is an important aspect of managing rice planthoppers in the karst region, particularly in Guizhou, because the planthoppers might emigrate northward. The insecticide resistance management programs should be implemented with international cooperative measures in Guizhou and in the larger rice-planting area of Southeast Asia. Additionally, rice growers should be trained in the rational use of insecticides (Xia et al. 2014).

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