Crop Protection 94 (2017) 106–114

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

The role of detoxifying enzymes in field-evolved resistance to nitenpyram in the brown planthopper *Nilaparvata lugens* in China

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ARTICLE INFO

Article history:
Received 19 October 2016
Received in revised form
17 December 2016
Accepted 20 December 2016
Available online 28 December 2016

Keywords:
Nilaparvata lugens (stål)
Nitenpyram
Insecticide resistance
Detoxification enzyme
Correlation analysis

ABSTRACT

The brown planthopper, *Nilaparvata lugens*, is one of the most economically important rice crop pests in Asia, and has developed resistance to various insecticides from most chemical groups including neonicotinoid insecticides. At present, nitenpyram is the primary insecticide for *N. lugens* control in paddy fields. Thus, the susceptibility of *N. lugens* field populations to nitenpyram is of concern because of its extensive application. In the present study, the LC_{50} values and the activities of the detoxifying enzymes of fifty-eight representative field populations of *N. lugens* were determined. The results showed that LC_{50} values of field populations of *N. lugens* varied from 0.45 to 6.44 mg a. i./L, revealing that *N. lugens* has developed a moderate level of resistance (resistance ratio, RR = 2.4–33.9-fold) to nitenpyram. The activities of the detoxification enzymes including cytochrome P450 monooxygenase ($r = 0.394$, $P = 0.002$) and esterase ($r = 0.274$, $P = 0.037$), showed significant correlations with the log LC_{50} values for the field populations of *N. lugens*. Moreover, piperonyl butoxide (PBO) showed obvious synergism (synergism ratio, SR = 1.6–2.1-fold) in the collected field populations. Obvious regional variation in nitenpyram susceptibility was detected among the field populations of *N. lugens*, suggesting that nitenpyram resistance has occurred in field populations of *N. lugens* in China, and the detoxification enzyme cytochrome P450 monooxygenase is more likely to a contributing factor to nitenpyram resistance.

1. Introduction

The brown planthopper, *Nilaparvata lugens* (Stål), is one of the most economically important rice crop pests in China and many other parts of Asia (Heong et al., 2015; Liu et al., 2015). It causes damage not only by directly feeding and ovipositing on rice stems but also by transmitting grass cluster dwarf virus and tooth dwarf virus, which together pose an additional threat to rice (Cabauatan et al., 2009; Lou and Cheng, 2011; Zhang et al., 2014). In 2005, China lost approximately 2.5 million tons of rice due to outbreaks of *N. lugens*, Likewise, in early 2012, China's southwestern provinces lost approximately 10 million tons of rice due to large planthopper outbreaks (Heong et al., 2015). Currently, an average of 1 million tons of paddy rice is lost annually (Heong et al., 2015). The damage of rice planthoppers to the rice crop is so severe that this species has been cited as a threat to global food security (Heong et al., 2015). Insecticides are considered the most important and reliable tool to prevent planthopper damage (Zhang et al., 2014; Liu et al., 2015). According to the Arthropod Pesticide Resistance Database (APRD), *N. lugens* has evolved resistance to 31 conventional insecticides used against *N. lugens* with 402 cases of insecticide resistance due to over-reliance on chemical insecticides for *N. lugens* management (APRD, 2016).

Nitenpyram is a neonicotinoid insecticide possessing a thiazolyl ring and was developed and commercialized by the Takeda Agro Company, Ltd. in 1995 (Elbert et al., 2008; Jeschke and Nauen, 2008; Jeschke et al., 2011). According to the mode of action classification of the Insecticide Resistance Action Committee (IRAC), the target of nitenpyram is the nicotinic acetylcholine receptor (nAChR), which plays an important role in the mediation of fast excitatory synaptic transmission in the insect central nervous system (CNS) (Vo et al., 2016). The characteristics of nitenpyram include a good systemic action and high insecticidal activity against sucking insect pests in the orders Hemiptera and Thysanoptera (Zhang, 1997; Wollweber and Tietjen, 1999; Elbert et al., 2008). In recent years, nitenpyram

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http://dx.doi.org/10.1016/j.cropro.2016.12.022
0261-2194/

has been one of the most important insecticides in rice protection (Wang et al., 2008; Zhang et al., 2014). Previous studies have shown that field populations of *N. lugens* remained susceptible to nitenpyram in 2007, 2011 and 2012 (Wang et al., 2008; Zhang et al., 2014). By contrast, the Arthropod Pesticide Resistance Database (APRD) records field populations of other pests, such as *Aphis gossypii* Glover, *Bemisia tabaci* Gennadius, *Leptinotarsa decemlineata* Say, *Musca domestica* Linnaeus, *Oxycarenus hyalinipennis* Costa and *Phenacoccus solenopsis* Tinsley, as having developed resistance to nitenpyram (Mota-Sanchez et al., 2006; Yuan et al., 2012; Matsuura and Nakamura, 2014; Abbas et al., 2015; Saddiq et al., 2015; Ullah et al., 2016).

Insecticide resistance often results from physiological changes that lead to the increased activity of detoxification enzymes such as esterases, glutathione *S*-transferases, and cytochrome P450 monooxygenases (Vontas et al., 2000, 2001, 2002; Heckel, 2012). Up-regulation of these detoxifying enzymes is the most common resistance mechanism (Heckel, 2012). Moreover, the enhanced activity of these detoxification processes can confer cross-resistance to insecticides that have the same mode of action or even to those with other modes of action (Lu et al., 2008; Mitchella et al., 2012; Zhang et al., 2016). Thus, studies on the mechanisms of resistance may provide useful information for pest resistance management.

Monitoring nitenpyram resistance in *N. lugens* and identifying the mechanisms conferring resistance to nitenpyram are essential for the efficient management of *N. lugens* resistance with the continued and extensive use of nitenpyram. In the present study, the rice-stem dipping method was used to assess the current status of nitenpyram resistance in field populations of *N. lugens* collected in eight Chinese provinces from 2011 to 2015, and detoxification enzymes were also assessed for their potential role in the development of resistance to nitenpyram in *N. lugens*.

2. Materials and methods

2.1. Insecticide and synergists

The insecticide nitenpyram (96%, technical grade, CAS 150824-47-8) was purchased from Hubei Kangbaotai Fine-Chemicals Co., Ltd. Triphenyl phosphate (TPP, 99%, CAS 115-86-6), diethyl maleate (DEM, 97%, CAS 141-05-9) and piperonyl butoxide (PBO, 90%, CAS 51-03-6) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Insect

Fifty-eight field populations of *N. lugens* were collected from eight provinces in China from 2011 to 2015 (Table 1). The collected insects were reared on rice seedlings at 27 ± 1 °C under 70%–80% relative humidity and a 16-h light/8-h dark photoperiod. The field-collected *N. lugens* were mated, and the third-instar nymphs of the first (F_1) generation were used for the bioassays. The third-instar nymphs of the second (F_2) generation of the nitenpyram-resistant field populations collected in 2015 were used for the synergism experiments. The LC_{50} value of the susceptible baseline for nitenpyram against *N. lugens* was established in the present study using a susceptible strain of *N. lugens*, which had been collected from a rice paddy at the Hunan Academy of Agricultural Sciences and reared on rice seedlings in the laboratory without exposure to any insecticide for more than 10 years.

2.3. Bioassay

Bioassays were performed with third-instar nymphs of the first (F_1) generation of *N. lugens* using a previously described rice-stem dipping method (Wang et al., 2008). Briefly, a nitenpyram stock solution was prepared by dissolving nitenpyram in double-distilled water containing 0.1% Triton X-100 (v/v). Rice plants at the tillering to early booting stage were pulled from the soil, washed thoroughly, cut to a length of approximately 10 cm including the roots, and air dried. Three rice stems were grouped together and immersed in the appropriate insecticide solution for 30 s and then air-dried at room temperature for at least 30 min. They were then wrapped with water-impregnated cotton and placed into 500 mL plastic cups (one group of three stems per cup). Third instar nymphs were collected with a homemade aspirating device, and fifteen nymphs were transferred into each cup. There were three replicates for each dose (concentration) and 6–9 doses for each insecticide. The control rice stems were treated with the 0.1% Triton X-100 water solution only. All treatments were maintained at 27 ± 1 °C under 70%–80% relative humidity and a 16-h light/8-h dark photoperiod. Mortality was assessed after exposure to nitenpyram for 96 h. The nymphs were considered dead if they were unable to move after a gentle prodding with a fine brush.

For the synergism analysis, rice seedlings and nymphs were sprayed with 100 mg/L aqueous solution of each synergist (PBO, DEM, and TPP) 12 h before the nitenpyram treatment.

2.4. Enzyme assays

To determine the cytochrome P450 monooxygenase, esterase, and glutathione *S*-transferase activities of *N. lugens* field populations, 50 nymphs of *N. lugens* from each population were homogenized on ice in 1000 μL of 0.1 M sodium phosphate buffer, pH 7.0, containing 1 mM EDTA, 1 mM dithiothreitol (DTT), 1 mM phenylthiourea, 1 mM PMSF, and 20% glycerol (Han et al., 2015). The homogenates were then centrifuged at 15,000×*g* for 20 min at 4 °C. The supernatants were harvested and stored at −80 °C until use, and the protein concentrations were determined using the Bio-Rad Protein Assay Kit.

Esterase activity was determined as previously described with slight modifications (Asperen, 1962). In brief, 200 μL of the assay mixture was pipetted into a 96-well plate that contained 2 μL of α-naphthyl acetate substrate (0.2 mM) and 10 μL of diluted enzyme solution in sodium phosphate buffer (0.2 M, pH 7.2). The mixture was then incubated at 37 °C for 15 min, the reaction was stopped by the addition of the colorimetric reagent FAST Blue B, and absorbance was measured with a microplate reader (Bio-Rad) at 600 nm.

Glutathione *S*-transferase activity was assessed using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate using a previously described method (Xu et al., 2014). Briefly, the 1000 μL reaction mixture consisted of 30 μL of 30 mM CDNB substrate solution, 30 μL of 30 mM GSH, and 50 μL of the diluted enzyme solution in sodium phosphate buffer (0.1 M, pH 7.5). The absorbance was measured using an ultraviolet spectrophotometer (Shimadzu UV-1800) at 340 nm for 5 min with a read interval of 30 s.

Cytochrome P450 monooxygenase activity was determined by *p*-nitroanisole (p-NA) as the substrate using a previously described method (Mayer et al., 1977; Wen et al., 2009). One hundred microliters of 2 mM p-NA, 10 μL of 9.6 mM NADPH, and 90 μL of the diluted enzyme solution in sodium phosphate buffer (0.1 M, pH 7.8) were combined. The mixture was pipetted into a 96-well plate and was incubated at 34 °C for 30 min with shaking, and the absorbance was recorded using a microplate reader (Bio-Rad) at 405 nm.

2.5. Data analysis

The mortality data were corrected using Abbott's formula. The LC_{50} values, 95% confidence intervals, and slopes were calculated by probit analysis (Finney, 1971). The resistance ratio (RR) was

Table 1
Sampling information for *Nilaparvata lugens* from the field.

Population	Location	Collection date	Site	Insect stage
NN-2011	Nanning, Guangxi	Oct 10, 2011	23.17° N, 108.30° E	nymphs and adults
NN-2012	Nanning, Guangxi	Oct 12, 2012	23.17° N, 108.30° E	nymphs and adults
NN-2013	Nanning, Guangxi	Oct 3, 2013	23.17° N, 108.30° E	nymphs and adults
NN-2014	Nanning, Guangxi	Oct 13, 2014	23.17° N, 108.30° E	nymphs and adults
NN-2015	Nanning, Guangxi	Oct 14, 2015	23.17° N, 108.30° E	nymphs and adults
YL-2011	Yulin, Guangxi	Oct 11, 2011	22.64° N, 110.18° E	nymphs and adults
YL-2012	Yulin, Guangxi	Oct 12, 2012	22.64° N, 110.18° E	nymphs and adults
YL-2013	Yulin, Guangxi	Oct 3, 2013	22.64° N, 110.18° E	nymphs and adults
YL-2014	Yulin, Guangxi	Oct 13, 2014	22.64° N, 110.18° E	nymphs and adults
GD-2011	Shaoguan, Guangdong	Oct 12, 2011	22.64° N, 110.18° E	nymphs and adults
GD-2012	Shaoguan, Guangdong	Oct 13, 2012	24.64° N, 113.64° E	nymphs and adults
GD-2013	Shaoguan, Guangdong	Oct 6, 2013	24.64° N, 113.64° E	nymphs and adults
GD-2014	Shaoguan, Guangdong	Oct 15, 2014	24.64° N, 113.64° E	nymphs and adults
GD-2015	Shaoguan, Guangdong	Oct 13, 2015	24.64° N, 113.64° E	nymphs and adults
HN-2013	Ningxiang, Hunan	Sept 28, 2013	28.23° N, 112.50° E	nymphs and adults
HN-2014	Ningxiang, Hunan	Sept 31, 2014	28.24° N, 112.53° E	nymphs and adults
HN-2015	Ningxiang, Hunan	Sept 2, 2015	28.24° N, 112.53° E	nymphs and adults
JX-2013	Nanchang, Jiangxi	Sept 23, 2013	28.64° N, 115.57° E	nymphs and adults
JX-2014	Nanchang, Jiangxi	Sept 30, 2014	28.72° N, 115.65° E	nymphs and adults
JX-2015	Nanchang, Jiangxi	Sept 26, 2015	28.72° N, 115.65° E	nymphs and adults
FY-2011	Fuyang, Zhejiang	Sept 22, 2011	30.00° N, 119.85° E	nymphs and adults
FY-2012	Fuyang, Zhejiang	Sept 26, 2012	30.00° N, 119.85° E	nymphs and adults
FY-2013	Fuyang, Zhejiang	Sept 22, 2013	29.99° N, 119.89° E	nymphs and adults
FY-2014	Fuyang, Zhejiang	Sept 27, 2014	30.04° N, 119.83° E	nymphs and adults
FY-2015	Fuyang, Zhejiang	Sept 24, 2015	30.04° N, 119.83° E	nymphs and adults
JS-2011	Wuxi, Jiangsu	Sept 23, 2011	31.87° N, 120.27° E	nymphs and adults
JS-2012	Wuxi, Jiangsu	Sept 27, 2012	31.87° N, 120.27° E	nymphs and adults
JS-2013	Wuxi, Jiangsu	Sept 23, 2013	31.87° N, 120.27° E	nymphs and adults
JS-2014	Wuxi, Jiangsu	Sept 28, 2014	31.87° N, 120.27° E	nymphs and adults
JS-2015	Wuxi, Jiangsu	Sept 25, 2015	31.87° N, 120.27° E	nymphs and adults
AH-2013	Hefei, Anhui	Sept 24, 2013	32.48° N, 117.17° E	nymphs and adults
AH-2014	Hefei, Anhui	Sept 29, 2014	31.99° N, 117.23° E	nymphs and adults
AH-2015	Hefei, Anhui	Sept 24, 2015	31.14° N, 117.12° E	nymphs and adults
WX-2012	Wuxue, Hubei	Sept 8, 2012	30.13° N, 115.59° E	nymphs and adults
WX-2013	Wuxue, Hubei	Sept 13, 2013	30.12° N, 115.60° E	nymphs and adults
WX-2015	Wuxue, Hubei	Sept 22, 2015	30.12° N, 115.60° E	nymphs and adults
TC-2011	Tongcheng, Hubei	Sept 8, 2011	29.27° N, 113.83° E	nymphs and adults
TC-2012	Tongcheng, Hubei	Sept 4, 2012	29.27° N, 113.83° E	nymphs and adults
TC-2013	Tongcheng, Hubei	Sept 15, 2013	29.27° N, 113.83° E	nymphs and adults
TC-2014	Tongcheng, Hubei	Sept 12, 2014	29.27° N, 113.83° E	nymphs and adults
TC-2015	Tongcheng, Hubei	Sept 29, 2015	29.25° N, 113.83° E	nymphs and adults
XG-2012	Xiaogan, Hubei	Sept 9, 2012	31.27° N, 113.84° E	nymphs and adults
XG-2013	Xiaogan, Hubei	Sept 17, 2013	31.27° N, 113.84° E	nymphs and adults
XG-2014	Xiaogan, Hubei	Sept 15, 2014	31.27° N, 113.84° E	nymphs and adults
XG-2015	Xiaogan, Hubei	Sept 23, 2015	31.27° N, 113.84° E	nymphs and adults
TM-2011	Tianmen, Hubei	Sept 15, 2011	30.42° N, 113.49° E	nymphs and adults
TM-2012	Tianmen, Hubei	Sept 18, 2012	30.42° N, 113.49° E	nymphs and adults
TM-2013	Tianmen, Hubei	Sept 20, 2013	30.45° N, 113.45° E	nymphs and adults
TM-2014	Tianmen, Hubei	Sept 23, 2014	30.45° N, 113.46° E	nymphs and adults
TM-2015	Tianmen, Hubei	Sept 14, 2015	30.45° N, 113.46° E	nymphs and adults
ZY-2012	Zaoyang, Hubei	Sept 20, 2012	31.98° N, 112.76° E	nymphs and adults
ZY-2013	Zaoyang, Hubei	Sept 10, 2013	31.83° N, 112.78° E	nymphs and adults
ZY-2014	Zaoyang, Hubei	Sept19, 2014	31.88° N, 112.77° E	nymphs and adults
ZY-2015	Zaoyang, Hubei	Sept 28, 2015	31.88° N, 112.77° E	nymphs and adults
ZJ-2012	Zhijiang, Hubei	Sept 13, 2012	30.26° N, 111.55° E	nymphs and adults
ZJ-2013	Zhijiang, Hubei	Sept 21, 2013	30.26° N, 111.55° E	nymphs and adults
ZJ-2014	Zhijiang, Hubei	Sept 24, 2014	30.26° N, 111.55° E	nymphs and adults
ZJ-2015	Zhijiang, Hubei	Sept 22, 2015	30.26° N, 111.55° E	nymphs and adults

calculated by dividing the LC_{50} value of a field population by the corresponding LC_{50} value of the susceptible baseline (Table 2). The classification of the resistance levels was determined according to Shao et al. (2013). with resistance indicated by the RR (resistance ratio): $\leq$ 5-fold being classified as susceptible, RR = 5–10-fold as a low level of resistance, RR = 10 – 100-fold as a moderate level of resistance, and RR > 100-fold as a high level of resistance. Correlations between the variables were calculated using the Pearson method in the SPSS Statistics software package. $P < 0.05$ indicated statistical significance. The partial LC_{50} values of nitenpyram against the field populations of *N. lugens* collected from 2011 to 2012 were cited from Zhang et al. (2014), and partial data for the activities of esterase (EST), glutathione *S*-transferase (GST), and cytochrome P450 monooxygenase (P450) in field populations of *N. lugens* in 2014 were cited from Zhang et al. (2016).

3. Results

3.1. Resistance of field populations of N. lugens to nitenpyram

The LC_{50} values varied from 0.45 to 6.44 mg a. i./L, with a 14.3-fold variation (the highest LC_{50} value/the lowest LC_{50} value)

Table 2
Toxicity of nitenpyram to different field populations of *Nilaparvata lugens*.

Populations	Insect number	Slope (SE)	LC_{50} (95% CI) mg/L	χ^2 (df)	RR
Susceptibility	405	2.20 (0.37)	0.19 (0.17–0.22)	0.16 (3)	1.0
NN-2011[a]	480	2.03 (0.21)	0.63 (0.49–0.77)	1.34 (3)	3.3
NN-2012[a]	420	1.20 (0.23)	0.76 (0.59–0.78)	1.10 (2)	4.0
NN-2013	405	1.32 (0.16)	1.90 (1.32–2.82)	8.31 (3)	10.0
NN-2014	405	1.31 (0.33)	1.91 (1.31–3.94)	0.55 (2)	10.1
NN-2015	270	2.57 (0.32)	3.57 (2.94–4.39)	1.57 (2)	18.8
YL-2011[a]	480	2.41 (0.29)	0.52 (0.37–0.65)	8.79 (3)	2.7
YL-2012[a]	420	1.60 (0.20)	0.90 (0.67–1.31)	5.75 (3)	4.7
YL-2013	405	1.55 (0.19)	1.55 (1.14–1.98)	5.62 (3)	8.2
YL-2014	405	1.20 (0.15)	1.94 (1.42–2.71)	7.43 (4)	10.2
GD-2011[a]	360	1.23 (0.10)	1.13 (0.87–1.46)	4.70 (2)	5.9
GD-2012[a]	420	1.84 (0.20)	0.95 (0.74–1.25)	8.57 (3)	5.0
GD-2013	405	1.74 (0.16)	2.69 (2.18–3.28)	13.74 (3)	14.2
GD-2014	405	1.78 (0.21)	1.32 (0.90–1.75)	3.29 (3)	6.9
GD-2015	270	2.32 (0.28)	2.79 (2.25–3.40)	1.32 (2)	14.7
HN-2013	405	2.50 (0.37)	0.88 (0.54–1.20)	14.45 (3)	4.6
HN-2014	405	2.07 (0.23)	1.06 (0.81–1.33)	0.94 (4)	5.6
HN-2015	270	2.14 (0.27)	2.17 (1.22–3.59)	6.02 (2)	11.42
JX-2013	405	1.59 (0.15)	3.04 (2.33–3.86)	5.41 (4)	16.0
JX-2014	405	2.20 (0.23)	1.51 (1.22–1.86)	5.51 (4)	7.9
JX-2015	270	2.34 (0.34)	6.44 (5.23–8.45)	0.47 (2)	33.9
FY-2011[a]	480	1.82 (0.18)	0.58 (0.45–0.71)	3.51 (4)	3.0
FY-2012[a]	420	1.38 (0.16)	1.33 (0.98–1.92)	1.35 (3)	7.0
FY-2013	405	1.75 (0.17)	2.85 (2.19–3.61)	2.79 (3)	15.0
FY-2014	405	1.93 (0.20)	1.48 (1.17–1.84)	2.89 (4)	7.8
FY-2015	270	2.77 (0.39)	1.93 (1.56–2.33)	0.58 (2)	10.2
JS-2011[a]	480	1.52 (0.15)	0.69 (0.54–0.88)	1.12 (3)	3.6
JS-2012[a]	420	1.39 (0.16)	1.59 (1.17–2.34)	2.38 (2)	8.4
JS-2013	405	1.95 (0.21)	1.90 (1.46–2.44)	4.10 (3)	10.0
JS-2014	405	2.69 (0.36)	0.69 (0.54–0.84)	2.30 (3)	3.6
JS-2015	270	3.57 (2.71)	2.71 (2.29–3.32)	1.23 (2)	14.3
AH-2013	405	1.79 (0.31)	1.55 (1.16–2.05)	6.11 (4)	8.2
AH-2014	405	1.84 (0.28)	2.07 (1.61–2.80)	0.05 (3)	10.9
AH-2015	270	2.14 (0.27)	4.09 (3.02–5.58)	2.30 (2)	21.5
ZJ-2012	420	1.32 (0.19)	1.67 (1.23–2.33)	0.38 (3)	8.8
ZJ-2013	405	2.29 (0.24)	1.68 (1.27–2.09)	3.19 (3)	8.8
ZJ-2014	405	2.88 (0.35)	1.14 (0.94–1.37)	4.56 (3)	6.0
ZJ-2015	225	4.41 (0.75)	2.76 (1.83–4.08)	0.17 (1)	14.5
TC-2011[a]	480	1.54 (0.16)	0.45 (0.35–0.57)	1.92 (3)	2.4
TC-2012[a]	420	1.59 (0.13)	1.06 (0.79–1.43)	0.30 (3)	5.6
TC-2013	405	2.24 (0.23)	1.89 (1.59–2.42)	2.78 (3)	9.9
TC-2014	405	2.97 (0.38)	1.91 (1.54–2.27)	2.70 (4)	10.1
TC-2015	270	2.59 (0.42)	3.33 (1.93–6.02)	2.59 (2)	17.5
TM-2011[a]	480	1.47 (0.12)	0.51 (0.39–0.64)	0.89 (3)	2.7
TM-2012[a]	420	1.90 (0.22)	1.75 (1.33–2.52)	7.52 (3)	9.2
TM-2013	405	1.92 (0.18)	2.11 (1.71–2.65)	11.96 (4)	11.1
TM-2014	405	1.71 (0.24)	3.06 (2.19–5.10)	0.85 (3)	16.1
TM-2015	270	2.23 (0.30)	1.46 (0.92–2.13)	3.18 (2)	7.7
ZY-2012	420	1.18 (0.18)	2.18 (1.55–3.32)	0.51 (2)	11.5
ZY-2013	405	1.77 (0.22)	0.80 (0.47–1.14)	2.60 (3)	4.2
ZY-2014	405	1.69 (0.19)	0.96 (0.73–1.23)	4.09 (3)	5.1
ZY-2015	270	2.74 (0.30)	3.66 (3.08–4.40)	3.75 (2)	19.3
WX-2012	420	1.49 (0.21)	2.00 (1.50–2.81)	3.16 (3)	10.5
WX-2013	405	1.63 (0.18)	1.61 (1.16–2.09)	2.25 (4)	8.5
WX-2015	270	2.31 (0.29)	1.64 (1.33–2.03)	1.71 (2)	8.6
XG-2012	420	1.54 (0.24)	2.67 (1.96–4.13)	0.27 (3)	14.1
XG-2013	405	1.83 (0.18)	2.31 (1.80–2.88)	3.44 (3)	12.2
XG-2014	405	1.47 (0.25)	2.05 (1.51–2.94)	0.43 (4)	10.8
XG-2015	270	2.01 (0.27)	3.88 (3.07–4.92)	1.26 (2)	20.4

[a] These data were cited from Zhang et al., 2014.

among the field populations of *N. lugens* (Table 2). The resistance ratio ranged from 2.4-fold to 33.9-fold (Table 2), showing a fairly heterogeneous response among the populations. The field population from Jiangxi in 2015 showed the highest resistance ratio to nitenpyram (RR = 33.9-fold), whereas the Tongcheng population in 2011 showed the lowest resistance ratio (RR = 2.4-fold) (Table 2). More importantly, the resistance levels of *N. lugens* to nitenpyram showed an increasing trend in the period 2011–2015 (Fig. 1A and B).

3.2. Enzyme activity and pair-wise correlation analysis

The activities of esterase (EST), glutathione *S*-transferase (GST), and cytochrome P450 monooxygenase (P450) were differed significantly among the field populations of *N. lugens* from 2011 to 2015 (Table 3). The esterase activities varied from 1.29 ± 0.24 (WX-2013) to 9.08 ± 1.41 (TC-2012) μmol/min/mg protein (Table 3), resulting in 7.0-fold variation among the *N. lugens* populations. The glutathione *S*-transferase activities also displayed 4.5-fold variation

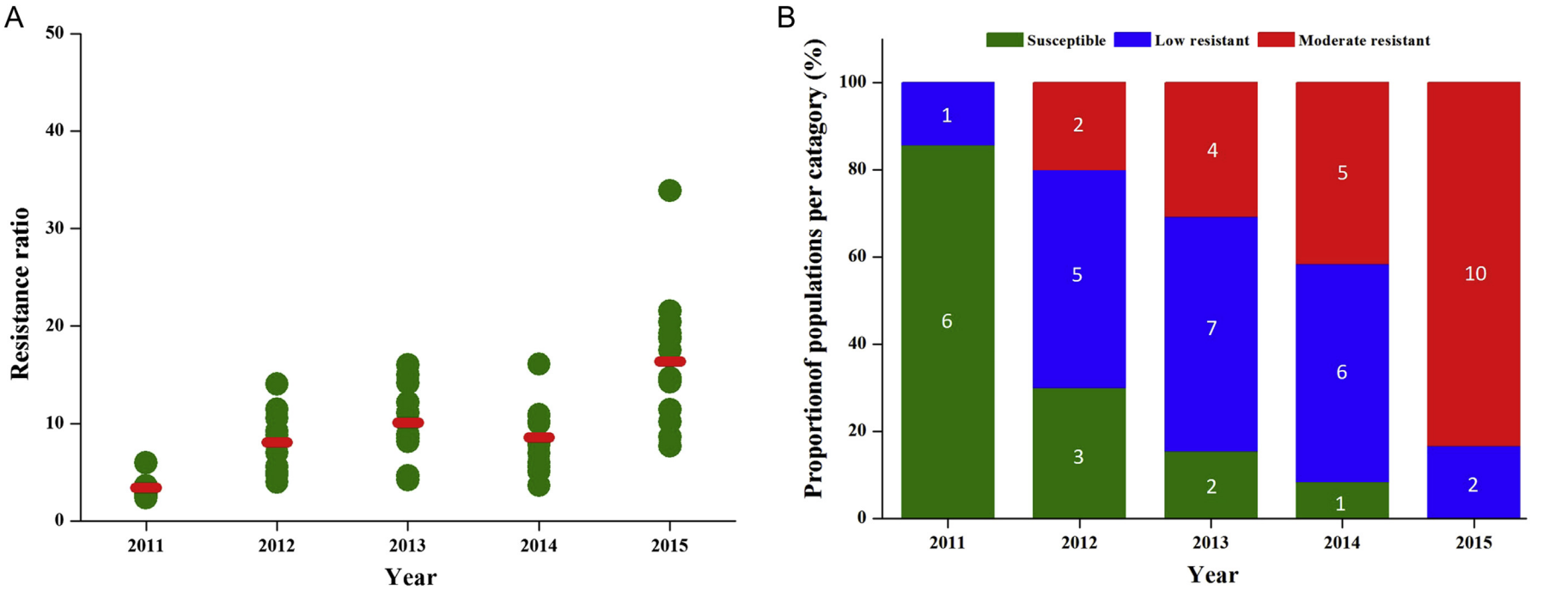

Fig. 1. Resistance levels of *Nilaparvata lugens* to nitenpyram. (A) Comparison of resistance levels of different field populations of *N. lugens* to nitenpyram from 2011 to 2015. Red horizontal lines across the scatter diagram represent the mean values of the resistance ratio of the different populations. (B) Trend in the variation in susceptibility to nitenpyram in field populations of *N. lugens*. The different colors represent the proportions of the different levels of resistance of *N. lugens* to nitenpyram each year from 2011 to 2015. Also shown is the number of field populations of *N. lugens* surveyed for nitenpyram resistance annually from 2011 to 2015 in each category. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Detoxifying enzyme activities of different field populations of *Nilaparvata lugens*.

Populations	Esterase μmol/min/mg protein	Glutathione *S*-transferase nmol/min/mg protein	Cytochrome P450 monooxygenase nmol/min/mg protein
Susceptibility	0.51 ± 0.02	5.78 ± 0.13	0.32 ± 0.01
NN-2011	4.51 ± 0.33	10.51 ± 1.34	0.44 ± 0.03
NN-2012	2.06 ± 0.38	6.56 ± 0.22	0.84 ± 0.08
NN-2013	4.29 ± 0.26	8.71 ± 0.17	0.55 ± 0.07
NN-2014[a]	3.41 ± 0.15	7.25 ± 0.26	0.60 ± 0.04
NN-2015	4.38 ± 0.61	12.34 ± 1.89	1.12 ± 0.12
YL-2011	2.21 ± 0.40	9.33 ± 0.33	0.44 ± 0.09
YL-2012	5.74 ± 0.36	14.50 ± 0.42	0.91 ± 0.07
YL-2013	2.22 ± 0.23	6.74 ± 0.34	0.60 ± 0.04
YL-2014	1.64 ± 0.09	7.89 ± 0.63	0.60 ± 0.15
GD-2011	2.68 ± 0.33	11.63 ± 1.15	0.80 ± 0.06
GD-2012	2.08 ± 0.72	16.83 ± 0.34	1.06 ± 0.14
GD-2013	4.95 ± 0.44	9.95 ± 0.56	1.09 ± 0.34
GD-2014[a]	3.42 ± 0.07	8.59 ± 0.81	0.92 ± 0.22
GD-2015	3.25 ± 0.45	14.99 ± 1.53	1.37 ± 0.28
HN-2013	2.96 ± 0.27	6.06 ± 0.44	0.69 ± 0.07
HN-2014[a]	1.69 ± 0.07	8.00 ± 0.34	0.68 ± 0.19
HN-2015	5.31 ± 0.53	5.86 ± 0.60	0.94 ± 0.16
JX-2013	1.53 ± 0.34	13.81 ± 0.98	1.39 ± 0.14
JX-2014[a]	2.62 ± 0.01	7.13 ± 0.40	0.73 ± 0.10
JX-2015	6.56 ± 1.88	13.63 ± 0.77	1.36 ± 0.29
FY-2011	3.77 ± 0.88	4.70 ± 0.43	0.49 ± 0.05
FY-2012	6.47 ± 0.39	7.40 ± 0.56	0.75 ± 0.06
FY-2013	8.22 ± 0.61	10.43 ± 0.57	1.17 ± 0.22
FY-2014[a]	3.65 ± 0.20	8.74 ± 0.60	0.73 ± 0.01
FY-2015	3.99 ± 0.64	6.35 ± 0.46	1.41 ± 0.35
JS-2011	3.20 ± 0.45	6.82 ± 0.30	0.49 ± 0.06
JS-2012	1.59 ± 0.27	4.98 ± 0.39	0.56 ± 0.09
JS-2013	5.21 ± 0.72	4.42 ± 0.39	0.90 ± 0.09
JS-2014[a]	2.67 ± 0.01	6.72 ± 0.15	0.52 ± 0.05
JS-2015	7.29 ± 0.49	9.21 ± 0.20	0.68 ± 0.14
AH-2013	4.57 ± 0.54	6.45 ± 0.46	0.53 ± 0.04
AH-2014[a]	1.95 ± 0.17	7.50 ± 0.40	0.58 ± 0.09
AH-2015	6.19 ± 0.52	14.49 ± 1.54	0.76 ± 0.16
ZJ-2012	7.92 ± 0.95	15.28 ± 2.14	0.79 ± 0.09
ZJ-2013	5.33 ± 0.45	9.91 ± 0.94	1.35 ± 0.19
ZJ-2014[a]	3.49 ± 0.05	7.99 ± 0.19	0.83 ± 0.04
ZJ-2015	6.01 ± 0.72	19.94 ± 1.72	1.38 ± 0.28
TC-2011	5.39 ± 0.40	13.84 ± 2.06	0.64 ± 0.20
TC-2012	9.08 ± 1.41	12.15 ± 1.28	0.52 ± 0.03
TC-2013	5.77 ± 1.22	5.98 ± 0.96	0.49 ± 0.06
TC-2014	4.70 ± 0.15	6.24 ± 0.83	0.90 ± 0.01
TC-2015	2.85 ± 0.37	10.86 ± 0.91	0.56 ± 0.12
TM-2011	1.74 ± 0.27	8.20 ± 0.41	1.33 ± 0.41
TM-2012	6.00 ± 0.95	8.63 ± 0.44	0.56 ± 0.08
TM-2013	3.92 ± 0.16	9.82 ± 0.74	0.37 ± 0.03
TM-2014	2.67 ± 0.35	9.77 ± 0.20	0.92 ± 0.02
TM-2015	1.79 ± 0.20	6.58 ± 0.27	0.52 ± 0.11
ZY-2012	8.55 ± 0.55	12.37 ± 1.49	0.99 ± 0.07
ZY-2013	5.49 ± 0.58	9.23 ± 0.39	0.89 ± 0.07
ZY-2014	1.47 ± 0.13	7.27 ± 0.60	0.99 ± 0.03
ZY-2015	4.95 ± 0.39	11.09 ± 1.99	0.92 ± 0.04
WX-2012	1.89 ± 0.21	4.56 ± 0.51	0.73 ± 0.05
WX-2013	1.29 ± 0.24	5.63 ± 0.38	0.91 ± 0.12
WX-2015	4.09 ± 0.46	11.46 ± 2.47	0.65 ± 0.19
XG-2012	5.44 ± 0.51	5.39 ± 0.75	0.86 ± 0.09
XG-2013	4.55 ± 0.45	14.03 ± 2.00	1.15 ± 0.35
XG-2014	3.71 ± 0.16	7.49 ± 0.52	0.81 ± 0.01
XG-2015	4.75 ± 1.08	14.36 ± 1.30	1.20 ± 0.27

Results are shown as the mean ± standard error.
[a] These data were cited from Zhang et al. (2016).

among the *N. lugens* populations, which varied from 4.42 ± 0.39 (JS-2013) to 19.94 ± 1.72 (ZJ-2015) nmol/min/mg protein. The activities of cytochrome P450 monooxygenase ranged from 0.37 ± 0.03 (TM-2013) to 1.41 ± 0.35 (FY-2015) nmol/min/mg protein with 3.8-fold variation (Table 3). Furthermore, the pair-wise correlation analysis indicated significant positive correlations between the log LC_{50} values of nitenpyram and the activities of detoxifying enzymes including the activity of cytochrome P450 monooxygenase ($r = 0.394$, $P = 0.002$) and esterase ($r = 0.274$, $P = 0.037$) (Table 4).

3.3. Synergism of PBO to nitenpyram

To verify the biochemical mechanism of resistance to nitenpyram in field populations of *N. lugens*, the cytochrome P450 monooxygenase inhibitor PBO, the esterase inhibitor TPP, and the glutathione *S*-transferase inhibitor DEM were used for synergism

Table 4
Pair-wise correlation between nitenpyram toxicities and enzyme activities in *Nilaparvata lugens*.

	Esterase	Glutathione *S*-transferase	Cytochrome P450 monooxygenase
Nitenpyram	0.274[a] (P = 0.037)	0.248 (P = 0.061)	0.394[a] (P = 0.002)

[a] Significant correlation between LC_{50} values of nitenpyram and enzyme activity at the 95% significance level.

Table 5
Synergistic effects of PBO, DEM, and TPP on nitenpyram in field populations of *Nilaparvata lugens*.

Populations	RR	Treatments	Insect number	Slope (SE)	LC_{50} (95% CI) mg/L	χ^2	SR[a]
Susceptibility	1.0	Nitenpyram	405	2.20 (0.37)	0.19 (0.17–0.22)	0.16	–
		Nitenpyram + PBO	360	1.61 (0.24)	0.27 (0.20–0.36)	0.34	0.7
		Nitenpyram + DEM	360	1.60 (0.24)	0.25 (0.18–0.35)	0.49	0.8
		Nitenpyram + TPP	360	1.70 (0.20)	0.22 (0.17–0.22)	0.42	0.9
NN-2015	18.8	Nitenpyram	360	2.01 (0.26)	3.20 (2.54–4.23)	2.30	–
		Nitenpyram + PBO	360	1.35 (0.35)	1.84 (1.28–3.58)	6.07	1.7
		Nitenpyram + DEM	360	2.16 (0.26)	2.63 (2.12–3.33)	1.15	1.2
		Nitenpyram + TPP	360	1.80 (0.22)	2.42 (1.89–3.12)	9.92	1.3
JX-2015	33.9	Nitenpyram	315	1.74 (0.22)	4.09 (3.17–5.68)	2.29	–
		Nitenpyram + PBO	315	1.51 (0.23)	1.94 (1.46–2.64)	0.96	2.1
		Nitenpyram + DEM	315	1.25 (0.14)	3.38 (2.27–5.42)	6.84	1.2
		Nitenpyram + TPP	315	1.67 (0.19)	2.39 (1.87–3.07)	1.09	1.7
AH-2015	21.5	Nitenpyram	270	1.78 (0.23)	3.36 (2.61–4.59)	2.95	–
		Nitenpyram + PBO	270	2.19 (0.30)	1.73 (1.46–1.96)	0.50	1.9
		Nitenpyram + DEM	270	1.86 (0.24)	2.47 (2.11–2.89)	0.80	1.4
		Nitenpyram + TPP	270	1.56 (0.21)	2.58 (1.97–3.47)	4.79	1.3
TC-2015	17.5	Nitenpyram	360	2.22 (0.31)	2.91 (2.38–3.54)	0.82	–
		Nitenpyram + PBO	360	1.93 (0.34)	1.82 (1.58–2.09)	0.14	1.6
		Nitenpyram + DEM	360	2.32 (0.33)	2.05 (0.96–3.79)	7.18	1.4
		Nitenpyram + TPP	360	2.40 (0.31)	1.61 (1.08–2.32)	3.36	1.8
ZY-2015	19.3	Nitenpyram	360	1.45 (0.16)	2.97 (2.26–3.99)	4.11	–
		Nitenpyram + PBO	360	1.45 (0.14)	1.73 (1.36–2.22)	3.47	1.7
		Nitenpyram + DEM	360	1.28 (0.14)	2.25 (1.93–2.63)	1.12	1.3
		Nitenpyram + TPP	360	1.32 (0.15)	1.92 (1.72–2.14)	0.55	1.5

[a] Synergistic ratio: LC_{50} for nitenpyram alone/LC_{50} for nitenpyram with synergist.

assays in 5 field populations collected in 2015 with moderate levels of resistance to nitenpyram (Table 2, Table 5). PBO showed obvious synergism to nitenpyram in tested field populations with a synergistic ratio (SR) ranged from 1.6 to 2.1, and TPP also showed a little synergism with a synergistic ratio (SR) that ranged from 1.3 to 1.8 in 5 tested field populations (Table 5). In contrast, DEM had no obvious effect on the toxicities of nitenpyram in any of the tested populations (Table 5).

4. Discussion

N. lugens has become a major insect pest in paddy fields in China and the rest of Asia (Cabauatan et al., 2009; Heong et al., 2015). Chemical insecticides have continued to be the main tool for controlling this pest due to its high fecundity and long-distance migratory behavior (Heong et al., 2015; Liu et al., 2015). However, *N. lugens* can develop resistance to insecticides rapidly as a result of their misuse and overuse (Wang et al., 2008; Zhang et al., 2014, 2016; Heong et al., 2015; Liu et al., 2015). Thus, to achieve successful management of *N. lugens*, an understanding of the current status of the resistance levels of field populations of *N. lugens* to nitenpyram is critical. This study revealed shifts in nitenpyram resistance and the biochemical mechanism of resistance in field populations of *N. lugens* collected from eight Chinese provinces in the period 2011–2015, and this information will be valuable for the management of nitenpyram resistance and this insect pest.

Because *N. lugens* evolved a high level of resistance to imidacloprid in 2005, nitenpyram has been used to control field populations of *N. lugens* throughout China, solely or in the form of mixtures with other insecticides (Wang et al., 2008; Zhang et al., 2014). The current study demonstrated a wide range of variation (14.3-fold) in the susceptibility of *N. lugens* from different areas, and some populations had developed moderate levels of resistance to nitenpyram. Similarly, resistance to nitenpyram has been reported in field populations of other insect species (Mota-Sanchez et al., 2006; Basit et al., 2011; Yuan et al., 2012; Matsuura and Nakamura, 2014; Abbas et al., 2015; Saddiq et al., 2015; Ullah et al., 2016). In the first demonstrated case of nitenpyram resistance, a population of Colorado potato beetles (*Leptinotarsa decemlineata*) collected from Long Island, New York, USA, developed 10-fold resistance (Mota-Sanchez et al., 2006). Subsequently, resistance to nitenpyram has been observed in populations of *Aphis gossypii, Bemisia tabaci, Musca domestica, Phenacoccus solenopsis,* and *Oxycarenus hyalinipennis* (Mota-Sanchez et al., 2006; Basit et al., 2011; Yuan et al., 2012; Matsuura and Nakamura, 2014; Abbas et al., 2015; Saddiq et al., 2015; Ullah et al., 2016). Although the levels of nitenpyram resistance were low to moderate, a resistance risk assessment for nitenpyram showed that *N. lugens* could develop resistance to nitenpyram under continuous exposure (Li et al., 2013). Therefore, more attention should be given to the risk of resistance to nitenpyram.

Another important aspect of pest management is to know the mechanism that causes resistance to insecticides (Wang et al., 2013). Insecticide resistance often results from gene regulatory changes, which lead to the increased activity of detoxification enzymes such as esterase, glutathione *S*-transferase, and cytochrome P450 monooxygenases (Heckel, 2012). Up-regulation of these detoxifying enzymes is the most common resistance mechanism (Heckel, 2012). Previous studies have shown that neonicotinoid insecticide resistance is mediated by cytochrome P450 monooxygenases (Li and Han, 2007; Feng et al., 2010; Puinean et al., 2010; Bass et al., 2011; Ding et al., 2013; Garrood et al., 2016).

Further, there have been a few reports that esterase appeared to be responsible for neonicotinoid insecticide resistance (Li and Han, 2007; Feng et al., 2010). However, previous studies have not demonstrated that glutathione *S*-transferase plays a role in neonicotinoid insecticide resistance in insect species (Li and Han, 2007; Wen et al., 2009; Feng et al., 2010; Puinean et al., 2010; Bass et al., 2011; Ding et al., 2013; Garrood et al., 2016). In the present study, we found that the activity of the detoxification enzymes (esterase and cytochrome P450 monooxygenases) were significantly correlated with the log LC_{50} values of nitenpyram, indicating that these detoxification enzymes may be involved in the observed resistance of *N. lugens* to nitenpyram. More importantly, PBO showed obvious synergism in nitenpyram-resistant strains. Thus, these results suggested that detoxification enzymes including cytochrome P450 monooxygenases and esterase may be involved in the observed resistance of field populations of *N. lugens* to nitenpyram.

Nitenpyram has gradually become the main insecticide for the control of *N. lugens* in China because of its excellent efficacy and low environmental impact (Akayama and Minamida, 1999). The application of nitenpyram against *N. lugens* will undoubtedly become more widespread in future. The findings of our five-year survey of susceptibility to nitenpyram revealed that this insecticide is still effective in controlling *N. lugens* in the main rice producing regions of China. However, in this study, the biochemical mechanisms of nitenpyram resistance in field populations of *N. lugens* from China were also determined. These data are beneficial for nitenpyram resistance management. Indeed, early shifts in the susceptibility of *N. lugens* to nitenpyram have started to occur; therefore, an effective resistance management strategy (rotation or mixture with other insecticide in the field) should be implemented as early as possible to avoid or retard the further development of insect pest resistance to nitenpyram.

Competing interests

The authors have declared that no competing interests exist.

Acknowledgements

This work was supported by the National Key Technology R&D Program (2012BAD19B01), the National Natural Science Foundation of China (31471795), and the Special Fund for Agro-Scientific Research in the Public Interest (201203038 and 201503107).

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