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

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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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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