Pest Management Science *Pest Manag Sci* **64**:1278–1284 (2008)

Susceptibility to neonicotinoids and risk of resistance development in the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae)

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Abstract

BACKGROUND: In recent years, outbreaks of the brown planthopper, *Nilaparvata lugens* (Stål), have occurred more frequently in China. The objective of this study was to determine the susceptibility of *N. lugens* to neonicotinoids and other insecticides in major rice production areas in China.

RESULTS: Results indicated that substantial variations in the susceptibility to different insecticides existed in *N. lugens*. Field populations had developed variable resistance levels to neonicotinoids, with a high resistance level to imidacloprid (RR: 135.3–301.3-fold), a medium resistance level to imidaclothiz (RR: 35–41.2-fold), a low resistance level to thiamethoxam (up to 9.9-fold) and no resistance to dinotefuran, nitenpyram and thiacloprid (RR < 3-fold). Further examinations indicated that a field population had developed medium resistance level to fipronil (up to 10.5-fold), and some field populations had evolved a low resistance level to buprofezin. In addition, *N. lugens* had been able to develop 1424-fold resistance to imidacloprid in the laboratory after the insect was selected with imidacloprid for 26 generations.

CONCLUSION: Long-term use of imidacloprid in a wide range of rice-growing areas might be associated with high levels of resistance in *N. lugens*. Therefore, insecticide resistance management strategies must be developed to prevent further increase in resistance.

Keywords: *Nilaparvata lugens*; susceptibility; neonicotinoid; buprofezin; fipronil

1 INTRODUCTION

The brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), is an economically important insect on rice in Asia.[1] In recent years, *N. lugens* outbreaks have occurred more frequently in the Yangtze River Delta areas and in the south of China.[2] This monophagous pest causes severe damage to rice plants through direct sucking, ovipositing and virus disease transmission. Because of its highly adaptive capacity to changing cultural practices and high reproductive potential, frequent chemical treatments to every generation are necessary to bring the insect populations under control.[3,4]

Neonicotinoids belong to a new insecticide class, chloronicotinyl nitroguanidines, which act as a competitive inhibitor on nicotinic acetylcholine receptors in the central nervous system. Their systemic properties and long residual activity make them ideal insecticides against sucking pests.[5] Imidacloprid was registered in the early 1990s and soon became the primary means for controlling *N. lugens* in many rice-growing areas in China.[6] After that, nitenpyram and thiamethoxam were introduced in China to control *N. lugens* because of their high efficacy against some sucking pests.[7,8] Imidaclothiz was developed by Nantong Jiangshan Agrochemical Co. Ltd (Nantong, Jiangsu, China), and was registered for use on rice against *N. lugens* in 2002.[9] Dinotefuran and thiacloprid have not yet been registered in China to control *N. lugens*. However, dinotefuran was registered to control *N. lugens* in Thailand,[10] where the insects continue feeding in winter and become the major source for north-bound migration to China in the following year.[11]

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(*Received 5 February 2008; revised version received 14 May 2008; accepted 22 May 2008*)
DOI: 10.1002/ps.1629

Pest Manag Sci 1526–498X/2008/$30.00

Buprofezin, an insect growth regulator, was introduced in China to control *N. lugens* in the late 1980s.[12] Fipronil, a novel phenylpyrazole insecticide, is a recently introduced insecticide to control *N. lugens* and other rice insect pests in most rice production regions in China.[13] Currently, buprofezin and fipronil are the primary insecticides for controlling *N. lugens* owing to the decreased efficacy of imidacloprid (Diao CY, private communication). Conventional insecticide classes, such as organophosphates and carbamates, are still being used to control *N. lugens*. However, these insecticides are only recommended for limited use in rotation with neonicotinoids, buprofezin, fipronil and pymetrozine (a pyridine azomethrine insecticide) to control planthoppers.[14]

Owing to the long history of chemical applications, *N. lugens* has evolved resistance to four major classes of insecticides: organochlorines, organophosphates, carbamates and pyrethroids.[15–17] Distinct biological and behavioral characteristics of *N. lugens*, such as short development time, high fecundity and high dispersal capacity, have contributed to the development of resistance to these conventional insecticides.[18] Resistance monitoring is a key to ensuring a successful resistance management program. Early detection of changes in resistance/susceptibility can prompt adoption of alternative control measures to slow down resistance development.[19] This study was initiated to determine the current status of susceptibility in *N. lugens* to neonicotinoids, including imidacloprid, thiamethoxam, dinotefuran, nitenpyram, thiacloprid and imidaclothiz, and to other commonly used insecticides, such as buprofezin and fipronil, in rice production areas. Risk of resistance development to imidacloprid in *N. lugens* was also assessed.

2 MATERIALS AND METHODS

2.1 Insects

The susceptible strain (S) of *N. lugens* was obtained from Zhejiang Chemical Industrial Group Co. Ltd (Hangzhou, Zhejiang, China). It was originally collected in 1995 in a rice field near Hangzhou (Zhejiang, China), and the insects have been reared on insecticide-free hybrid rice seedlings (Shanyou 63) for approximately 120 generations in the laboratory.

To examine the susceptibility of *N. lugens* to insecticides in different rice-growing areas, six populations were collected in August 2006 and 2007 from Nanning (Guangxi), Fuqing (Fujian), Shanggao (Jiangxi), Hexian (Anhui), Jinhua (Zhejiang) and Nanjing (Jiangsu). Selection of the collection sites was based on the migration route of *N. lugens* from the southern to the north-eastern part of China, the importance of rice production and the history of insecticide applications in the areas. Approximately 800 adults and 500–600 nymphs were collected from each site and transported to the greenhouse on the campus of Nanjing Agricultural University. The same rice variety at tillering to booting stage was used for maintaining insect colonies and subsequent bioassays. The field-collected insects were mass mated, and the third-instar nymphs of F_1 progenies were used for bioassays.

To assess potential risk of resistance development in *N. lugens*, a laboratory colony was developed from a field population originally collected in 1993 from a rice field near Nanjing (Jiangsu, China). The colony of third-instar nymphs was treated with imidacloprid once every two generations using a rice seedling spray method[20] in the laboratory. After periodical selections with imidacloprid, the colony developed 200-fold resistance in 2005 (unpublished data). This colony with 200-fold resistance to imidacloprid was used as a starting colony (generation 0) in the present study for imidacloprid selection. The study lasted a total of 26 generations, and third-instar nymphs of every generation were treated with imidacloprid using the rice-stem dipping method.[21]

2.2 Insecticides

Technical imidacloprid (95%) and buprofezin (98.1%) were provided by Changlong Chemical Industrial Group Co. Ltd (Changzhou, Jiangsu, China). Nitenpyram (95%) and imidaclothiz (95%) were supplied by Nantong Jiangshan Agrochemical Co. Ltd (Nantong, Jiangsu, China). Technical thiamethoxam (97.2%) and dinotefuran $100\,\mathrm{g\,L^{-1}}$ SL were obtained from Syngenta Investment Co. Ltd. (Shanghai, China). Thiacloprid (97.75%) was supplied by Tianjing Xingguang Chemical Co. Ltd (Tianjing, China), and fipronil (87%) was provided by Bayer CropScience Hangzhou Co. Ltd (Hangzhou, Zhejiang, China). The technical products were formulated as emulsifiable concentrates (EC) by mixing with $100\,\mathrm{g\,L^{-1}}$ Triton X-100 and acetone for the laboratory assays.

2.3 Bioassay

Toxicities of neonicotinoids and all other insecticides were measured using the rice-stem dipping method.[21] Rice plants at tillering to booting stage were pulled out and washed thoroughly. Rice stems (about 10 cm length) with roots were cut and air dried to remove excess water. Three rice stems were grouped and dipped into the appropriate insecticide test solution for 30 s. Three replicates were used per dose, and 5–6 doses plus distilled water only as control were used for each chemical. After the rice stems had been air dried for approximately 1 h, moistened cotton was used to wrap the rice roots. The treated rice stems were then placed into a 500 mL plastic cup. Twenty third-instar nymphs of *N. lugens* were introduced into each plastic cup using a vacuum device. The treated insects were maintained at a temperature of 27 ± 1 °C with a 16:8 h light:dark photoperiod. Mortality was recorded after 96 h for all insecticide treatments, except for the 120 h mortality recorded for the buprofezin treatment. The nymphs were considered dead if they were unable to show movement after a gentle prod with a fine bristle.

2.4 Laboratory selection for resistance to imidacloprid

The same rice-stem dipping method as described in Section 2.3 was adopted for resistance selection of the laboratory colony. Rice stems were treated with imidacloprid and were transferred into a cage (57 × 57 × 92 cm). Approximately 1000 third-instar nymphs of every generation were treated with imidacloprid by the rice-stem dipping method and subsequently maintained at $27 \pm 1\,^{\circ}\mathrm{C}$ with a 16:8 h light:dark photoperiod for 4 days. Survivors were transferred to another cage containing fresh rice seedlings. The mortality for resistance selection was controlled to a range between 40 and 70% to ensure sufficient survivors to develop and reproduce sufficient progeny for subsequent insecticide selection (the treated concentration was similar to the LC_{50} value of each generation).

2.5 Data analyses

The mortality data were corrected using Abbott's formula[22] and analyzed by probit analysis using POLO-PC.[23] The resistance ratio (RR) was calculated by dividing the LC_{50} value of a field population by the corresponding LC_{50} value of the susceptible strain. Two LC_{50} values were considered to be significantly different if their 95% confidence intervals did not overlap. Based on the standard described by Shen and Wu,[24] resistance levels were classified as susceptible ($RR < 3$-fold), minor resistance ($RR = 3-5$-fold), low resistance level ($RR = 5-10$-fold), medium resistance level ($RR = 10-40$-fold), high resistance level ($RR = 40-160$-fold) and extremely high resistance level ($RR < 160$-fold).

3 RESULTS

3.1 Variations in dose response and resistance ratios to eight insecticides

Susceptibilities to neonicotinoids, buprofezin and fipronil in six field populations of *N. lugens* collected from six provinces (autonomous regions) were evaluated in 2006 and 2007. The dose response data of eight insecticides to the susceptible strain are listed in Table 1. Thiacloprid had the lowest activity [$LD_{50} = 13.5$ (10.60–17.70) mg AI L^{-1}] against the susceptible strain of *N. lugens*, which was significantly different from those of the other seven insecticides [LD_{50} ranging from 0.04 (0.03–0.05) for fipronil to 0.47 (0.25–0.61) mg L^{-1} for nitenpyram].

Pooled RR data from six populations indicated that substantially different resistance levels were developed in *N. lugens* to eight different insecticides. An extremely high resistance level was developed to imidacloprid (mean RR value 224.1-fold), a medium resistance level to imidaclothiz (RR 34.2-fold), low resistance levels to thiamethoxam and fipronil (RR 5.4- and 5.2-fold respectively) and a minor resistance to buprofezin (RR 4.0-fold). All of the populations were still very susceptible to thiacloprid, dinotefuran and nitenpyram ($RR < 3$-fold).

3.2 Variations in resistance ratios among populations for each insecticide

3.2.1 Neonicotinoids

High to extremely high resistance levels to imidacloprid (RR 135.3–301.3-fold) and medium to high resistance levels to imidaclothiz (RR 30.8–41.2-fold) were found in the field populations of *N. lugens* in 2006 and 2007 (Figs 1A and B). Most field populations developed a low resistance level to thiamethoxam (RR 5–7.7-fold), except Shanggao (Jiangxi), Jinhua (Zhejiang) and Nanjing (Jiangsu) populations, which were still susceptible or slightly resistant to the chemical in 2006 (Fig. 1C). However, all field populations of *N. lugens* were still susceptible to dinotefuran, nitenpyram and thiacloprid in 2007 ($RR < 3$-fold), although the resistance ratios to each insecticide were substantially different among six different field populations (Fig. 1D).

3.2.2 Buprofezin

The six populations of *N. lugens* collected from six different provinces (autonomous regions) were also examined for their susceptibilities to buprofezin in 2006 and 2007. The results showed that populations collected in 2006 from Nanning (Guangxi), Hexian (Anhui) and Nanjing (Jiangsu) and a population collected in 2007 from Nanjing developed low resistance levels to buprofezin (RR 5.6–9.9-fold). The other field populations maintained susceptibility or developed only minor resistance ($RR < 5$-fold) to the insecticide (Fig. 2A). Three populations collected from Nanning, Fuqing and Hexian showed a decrease,

Table 1. LC_{50} values of the susceptible strain of *Nilaparvata lugens* used as susceptibility baselines for neonicotinoids and other insecticides

Insecticide class	Insecticide	N^a	Slope (SE)	LC_{50} (mg AI L^{-1}) (95% CL)
Neonicotinoid	Dinotefuran	420	2.72 (0.23)	0.14 (0.10–0.18)
	Imidacloprid	420	2.51 (0.20)	0.08 (0.05–0.11)
	Imidaclothiz	420	2.10 (0.16)	0.33 (0.27–0.40)
	Nitenpyram	420	2.17 (0.18)	0.47 (0.25–0.61)
	Thiacloprid	420	1.35 (0.13)	13.50 (10.60–17.70)
	Thiamethoxam	420	2.18 (0.15)	0.11 (0.09–0.12)
Insect growth regulator	Buprofezin	420	4.25 (0.39)	0.08 (0.06–0.09)
Phenylpyrazole	Fipronil	420	2.15 (0.18)	0.04 (0.03–0.05)

[a] Number of insects tested.

DOI: 10.1002/ps

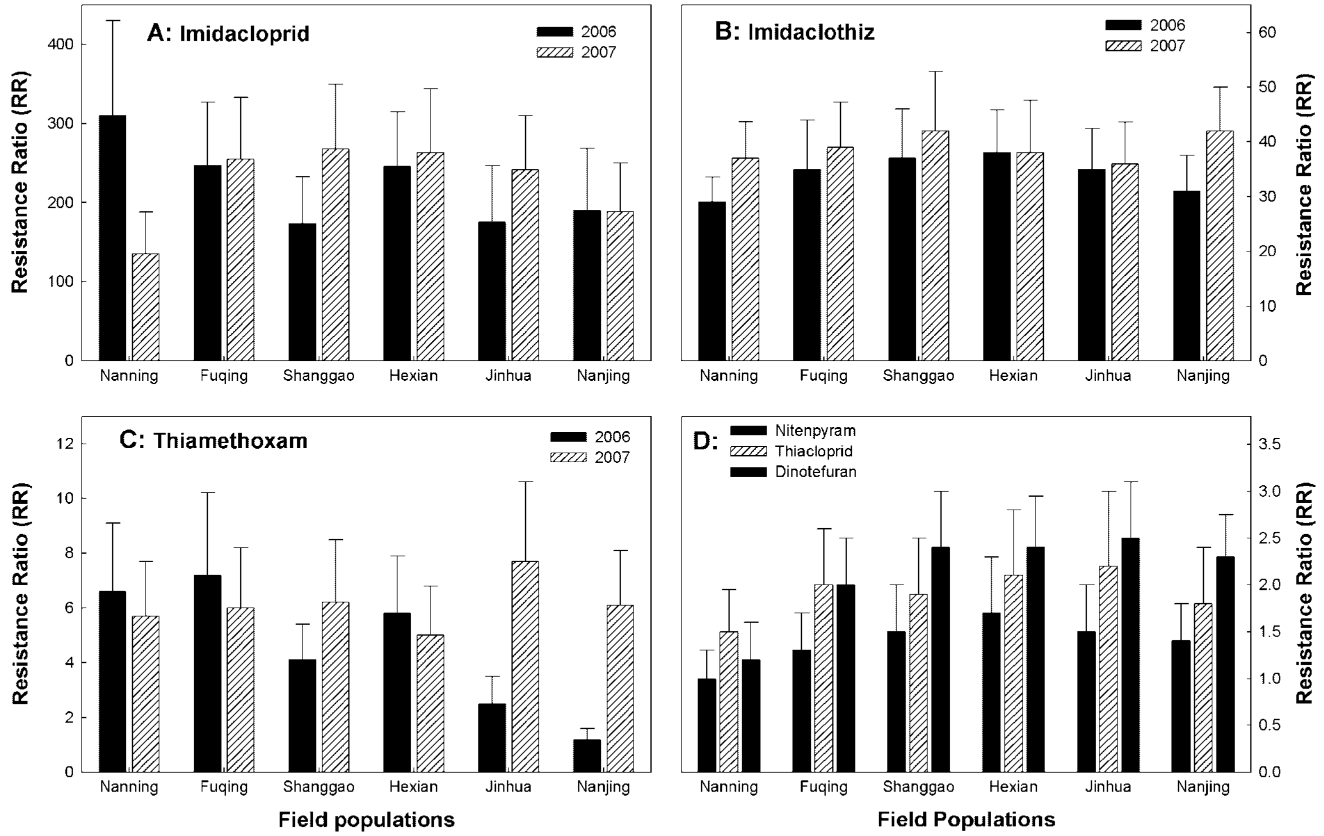

Figure 1. Resistance ratios in 2006 and 2007 to imidacloprid (**A**), imidaclothiz (**B**) and thiamethoxam (**C**), and resistance ratios in 2007 to nitenpyram, thiacloprid and dinotefuran (**D**) in six field populations of *Nilaparvata lugens*.

whereas the other three populations collected from Shanggao, Jinhua and Nanjing showed an increase in resistance ratios over the 2 year period. The resistance ratios were substantially different among the populations. The Fuqing population showed relatively low resistance ratios, while the Nanjing population had relatively high resistance ratios in both years (Fig. 2A).

3.2.3 *Fipronil*

Susceptibilities to fipronil in field populations of *N. lugens* collected from the six locations were evaluated in 2006 and 2007. The results showed that resistance ratios to the insecticide were also substantially different among the populations (Fig. 2B). A medium resistance level to fipronil (RR 10.5-fold) was found in the Nanjing (Jiangsu) population in 2007. In addition, a low resistance level was found in Nanning (Guangxi) in 2006 and Shanggao (Jiangxi) and Jinhua (Zhejiang) populations in 2007, while the other populations developed a minor resistance level to fipronil (RR 3–4.5-fold). All the populations, except the Nanning population, showed an increase in resistance ratios over the 2 year period (Fig. 2B).

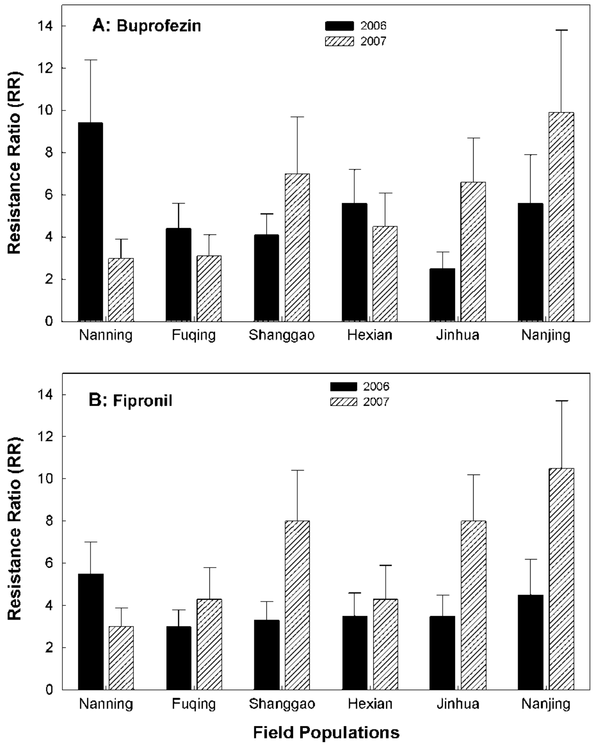

Figure 2. Resistance ratios to buprofezin (**A**) and fipronil (**B**) in 2006 and 2007 in six field populations of *Nilaparvata lugens*.

3.3 Resistance selection

The laboratory colony of *N. lugens* was selected continuously with imidacloprid for 26 generations in the laboratory. The results showed that the resistance level to imidacloprid increased by 7.1-fold over the 26-generation selection period. The insects increased RRs from 200.1-fold in the starting generation to 1360.2-, 1408.5- and 1423.9-fold after being selected for 24, 25 and 26 generations respectively (Fig. 3). The results

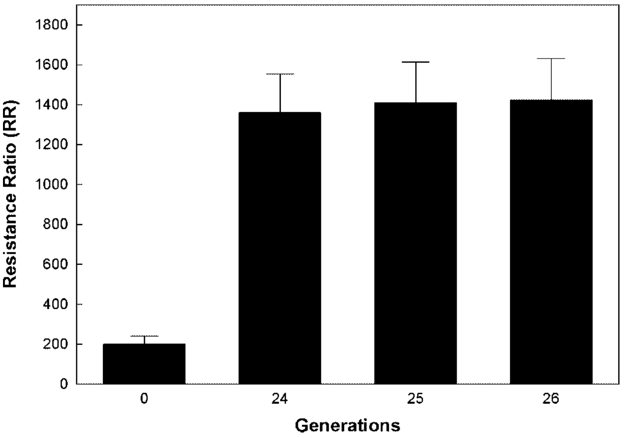

Figure 3. Resistance levels in generations 24 to 26 of *Nilaparvata lugens* after each generation was selected in the laboratory with imidacloprid for a total of 26 generations. Generation 0 was developed from a field population and was used as the resistance baseline before the selection started.

showed that continuous selection with imidacloprid could further increase the resistance level even when a high resistance level had already been developed in the colony.

4 DISCUSSION

In this study, six field populations of *N. lugens* were surveyed for their susceptibilities to three different classes of insecticide. The present bioassay results indicated that the susceptibilities of *N. lugens* to neonicotinoids and other classes of insecticides were potentially correlated with the selection pressures resulting from variable insecticide application histories and intensities in China. The results from this study basically agreed with the evolutionary theory of insecticide resistance, which is driven mainly by selection of insecticides.[25,26]

Currently, control of *N. lugens* relies almost exclusively on insecticides. Insecticide resistance development in the insect has become a serious issue. In the past, organophosphates, carbamates and buprofezin were the major insecticides for controlling *N. lugens.*[12] Since its introduction in China in the early 1990s, imidacloprid has been extensively used along with some new highly effective insecticides, such as fipronil and thiamethoxam.[14,27] However, since 2006 the use of imidacloprid as a means to control *N. lugens* has been temporarily suspended because its efficacy decreased significantly against the insect in 2005 in the Yangtze River Delta areas, including Jiangsu, Zhejiang, Anhui and other provinces (Diao CY, private communication). In addition, phasing out highly toxic organophosphate insecticides created a shortage of alternative insecticides and left farmers with no choice other than relying exclusively on a few insecticides, such as buprofezin and fipronil.[2] As a consequence of lack of diversity and misuse of the insecticides (such as improper increase in dose to pursue or maintain high efficacy), high selection pressure on the target insect might have prompted resistance development in field populations in many rice-growing areas.

The results from this study also indicate that the resistance to imidacloprid in *N. lugens* is widespread among the rice production areas in China. The author's laboratory selection study demonstrated that *N. lugens* had potential to develop very high levels of resistance (1423.9-fold in 26 generations), suggesting that intensive use of imidacloprid (applied to every generation) might be a risk, allowing field populations to develop high levels of resistance against this insecticide. The selection study also indicated that the insect could increase resistance further, even when a high resistance level had already been developed in a population.

Other neonicotinoids, such as thiamethoxam, nitenpyram and imidaclothiz, are currently deployed in rice-growing areas for *N. lugens* control. Several neonicotinoids, such as thiamethoxam, dinotefuran and nitenpyram, were more efficient in controlling *N. lugens* than the conventional insecticides (i.e. organophosphates and carbamates). These results correlated with those of previous studies which also showed a high efficacy of some neonicotinoids against *N. lugens.*[28,29] However, field populations of *N. lugens* have developed a medium resistance level to imidaclothiz and a low resistance level to thiamethoxam. The present results indicate that the development of resistance management strategies is urgently needed to control resistant populations of *N. lugens.*

Buprofezin remained an important chemical for *N. lugens* control until the early 1990s when imidacloprid was introduced to control the insect.[30] Currently, buprofezin is again recommended as one of the main alternatives for replacing methamidophos. Some field populations of *N. lugens* have developed low levels of resistance to buprofezin. This might be associated with widespread and long-term usage of buprofezin to control *N. lugens*. Similar situations of increased buprofezin resistance due to extensive and intensive applications have also been observed in many other insects, such as *Bemisa tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood).[31,32]

Fipronil has been applied extensively in Jiangsu and Zhejiang provinces since approximately 1997. Owing to the development of high-level resistance to imidacloprid in *N. lugens* and the banning of highly toxic organophosphates by the Ministry of Agriculture in China, fipronil has become one of the primary insecticides for controlling *N. lugens* and other insects on rice in most of the rice production areas. *Nilaparvata lugens* remained susceptible to slightly resistant to fipronil until 2006, and then, in 2007, the field population from Nanjing developed medium levels of resistance to the chemical. Currently, although most other field populations of *N. lugens* maintain a minor to low level of resistance, the insect may become more resistant to fipronil if the insecticide is used continuously and widely without implementing proper resistance management strategies.

Although this study provided a comprehensive survey of the susceptibilities and/or resistance levels to neonicotinoids and other insecticides and a potential connection between resistance severity and insecticide application intensity, several issues need to be addressed before a successful resistance management program can be developed and implemented. Firstly, it is important to study the nature of the resistance to determine whether it is dominant or recessive, so that resistance management strategies can be developed accordingly. Secondly, resistance mechanisms need to be clarified. Although enhanced oxidative detoxification[27] and a target-site mutation conferring reduced sensitivity to imidacloprid have been implicated,[33] imidacloprid resistance mechanisms are still far from being clearly understood. Thirdly, insecticide resistance management tactics need to be strengthened, including an extensive resistance monitoring program, regulation and recommendation of application frequencies and alternating and rotating imidacloprid with insecticides without cross-resistance. These strategies can be effective tools for minimizing further development and spread of insecticide resistance. Finally, integrated pest management (IPM) programs need to be developed. Along with chemical control, other strategies, such as biological control and cultural practices, should be integrated into the IPM programs.

ACKNOWLEDGEMENTS

The authors are grateful to Dr Xinzhi Ni (USDA-ARS, Tifton, GA), Dr Xuming Liu (Kansas State University, Manhattan, KS), Dr Yigen Chen (University of Georgia, Tifton, GA) and Sandy West (USDA-ARS, Stoneville, MS) for valuable suggestions and critical review of the early version of the manuscript. The study was partially supported by the Research Project of the Ministry of Agriculture in China for Replacing High-toxicity Pesticides.

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