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Dynamics of imidacloprid resistance and cross-resistance in the brown planthopper, *Nilaparvata lugens*

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Abstract

Extensive use of imidacloprid for suppressing the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), has placed heavy selection pressure on the target insect. A systematic study was carried out to determine imidacloprid resistance dynamics and cross-resistance. Data collected from a 3-year study (2005–2007) showed that in 2005, the resistance levels in Nanning (Guangxi), Haiyan (Zhejiang), and Nanjing and Tongzhou (Jiangsu) populations ranged from 200- to 799-fold compared with the susceptible strain. However, the resistance levels decreased to 135- to 233-fold in 2007, after reduced application of the chemical. A laboratory population was challenged with imidacloprid in successive generations. After 23 generations, the resistance ratio had increased from 200- to 1 298-fold. Continuous selection with imidacloprid could increase the resistance level even more than has already been developed in the population. Stopping selection with imidacloprid led to a rapid decrease of resistance from 759- to 114-fold after 17 generations. Resistance levels then became stable without decreasing any further. A similar result was also obtained from a study involving a field population (resistance ratio = 625-fold) collected from Tongzhou. At first, the population showed a rapid decrease in resistance right after imidacloprid selection was stopped, and then the resistance stabilized at a level of 105–129-fold. More interestingly, resistance increased again when selection was resumed. In addition, the resistant strain selected with imidacloprid showed substantial cross-resistance to imidaclothiz, thiacloprid, and acetamiprid, and slight levels of cross-resistance to dinotefuran and thiamethoxam, but no obvious cross-resistance to nitenpyram, buprofezin, and fipronil. The information from this study is valuable for formulating resistance-management strategies against *N. lugens*.

Introduction

The brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), is an economically important insect of rice in Asia (Heinrich, 1994). Hopperburn, caused by severe feeding damage, is a serious threat to rice yields in most rice-growing areas (Ding & Su, 2002). Currently, chemical control is still a major method for suppressing *N. lugens* (Endo & Tsurumachi, 2001). Intensive use of imidacloprid has prompted adaptation of *N. lugens* to the chemical. Since the introduction of DDT, *N. lugens* has developed resistance to almost every insecticide introduced for *N. lugens* control. Each newly introduced chemical provided effective control of *N. lugens* at first, then became less effective years later. New chemicals have

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been sought for better control of *N. lugens* because of these frequent chemical control failures (Kilin et al., 1981; Chung & Sun, 1983; Hirai, 1993).

Neonicotinoid insecticides, including imidacloprid, thiamethoxam, dinotefuran, nitenpyram, acetamiprid, thiacloprid, and imidaclothiz, are a well-established group of insecticides (Jeschke & Nauen, 2005). Many of these chemicals are still highly effective against most field populations of *N. lugens* (Tang et al., 2006). Imidacloprid was registered for controlling *N. lugens* on rice in the early 1990s. It quickly became the primary insecticide in many rice-growing areas in China because of its systemic nature and high efficacy against sucking insects (Sun et al., 1996). Continuous use of imidacloprid as a major insecticide for controlling *N. lugens* has resulted in a gradual decrease of efficacy against the pest. The application dose has had to be increased from 15 g a.i./ha in the 1990s to 60–120 g a.i./ha in 2005 in order to maintain effective control. However, in the Yangtze River Delta areas, such as Jiangsu, Zhejiang, Anhui, and other provinces, the control efficacy dropped from 95 to 60% even as application dose increased substantially (CY Diao, unpubl.). Although laboratory selection of *N. lugens* with imidacloprid has produced a highly resistant strain (Liu et al., 2003; Liu & Han, 2006), very little research has been done to survey and characterize imidacloprid resistance in field populations of *N. lugens*.

In an effort to understand the resistance mechanisms, researchers found that piperonyl butoxide synergized imidacloprid toxicity in green peach aphid [*Myzus persicae* (Sulzer)], cat flea [*Ctenocephalides felis* (Bouché)], house fly (*Musca domestica* L.), and tobacco whitefly [*Bemisia tabaci* (Gennadius)], suggesting that P450-mediated detoxification could be an important biochemical mechanism for imidacloprid resistance (Prabhaker et al., 1997; Wen & Scott, 1997; Richman et al., 1999; Choi et al., 2001; Nauen et al., 2002). Therefore, proper incorporation of these inhibitors with imidacloprid may help to abolish or suppress resistance in imidacloprid-resistant populations (Tang et al., 2006).

Cross-resistance is a potential risk that can limit the durability of an insecticide. Cross-resistance to various neonicotinoid insecticides was observed in field and laboratory-selected strains of *B. tabaci*, *Leptinotarsa decemlineata* (Say), and *Drosophila melanogaster* (Meigen) (Elbert & Nauen, 2000; Le Goff et al., 2003; Nauen & Denholm, 2005; Mota-Sanchez et al., 2006; Alyokhin et al., 2007). In these cases, reduced susceptibility to imidacloprid was linked to reduced susceptibility to other neonicotinoids, including thiamethoxam, acetamiprid, and nitenpyram. In *N. lugens*, cross-resistance to acetamiprid was also found in an imidacloprid-resistant strain developed in the laboratory (Liu et al., 2003). Therefore, research needs to be conducted to clarify whether cross-resistance also exists between imidacloprid and other neonicotinoids.

Susceptibility recovery in resistant populations is important for resistance management (Lan & Zhao, 2001). Numerous studies have shown that resistance can become relatively stable once insects have developed significant resistance, and that declines in insecticide resistance could be slow after the insecticide is no longer used, as was observed in the resistance of *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) and *Carpocapsa pomonella* (L.) to DDT, and *M. domestica* to isolan (Stone, 1962; Barnes & Moffit, 1963; Georghiou, 1964). In contrast, in some cases such as pyrethroid resistance in *Helicoverpa armigera* (Hübner) and triazophos resistance in *Chilo suppressalis* (Walker) (Wu et al., 1996; Qu et al., 2005), resistance was not very stable and tended to decline after use of the chemical ceased.

Nilaparvata lugens has developed significant resistance to imidacloprid in China since 2005 (JL Shen, unpubl.), and other neonicotinoids as well as the non-neonicotinoids buprofezin and fipronil are currently being implemented in rice-growing areas for insect control and resistance management. However, it was not clear whether the imidacloprid-resistant populations had already developed cross-resistance to other neonicotinoids, and non-neonicotinoids such as buprofezin and fipronil, and whether stopping or reducing imidacloprid applications would allow susceptibility to be recovered in the resistant field populations. In order to develop better resistance management strategies, this study was designed to examine the change in imidacloprid resistance under different selection pressures, and to investigate whether cross-resistance to other neonicotinoids and to buprofezin and fipronil existed in the imidacloprid-resistant strain.

Materials and methods

Insecticides

Imidacloprid [95% technical product (TC)], acetamiprid (97% TC), and buprofezin (98.1% TC) were provided by Changlong Chemical Industrial Group (Changzhou, Jiangsu, China), Nitenpyram (95% TC) and imidaclothiz (95% TC) by Nantong Jiangshan Agrochemical (Nantong, Jiangsu, China), Thiamethoxam (97.2% TC) and dinotefuran [10% soluble concentrate (SL)] by Syngenta Investment (Shanghai, China), Thiacloprid (97.75% TC) by Tianjing Xingguang Chemical (Tianjing, China), and Fipronil (87% TC) by Bayer Cropscience Hangzhou (Hangzhou, Zhejiang, China). For the laboratory assays, emulsifiable concentrates were prepared by mixing each insecticide (technical grade) with 10% (wt/vol) Triton-X-100 and acetone.

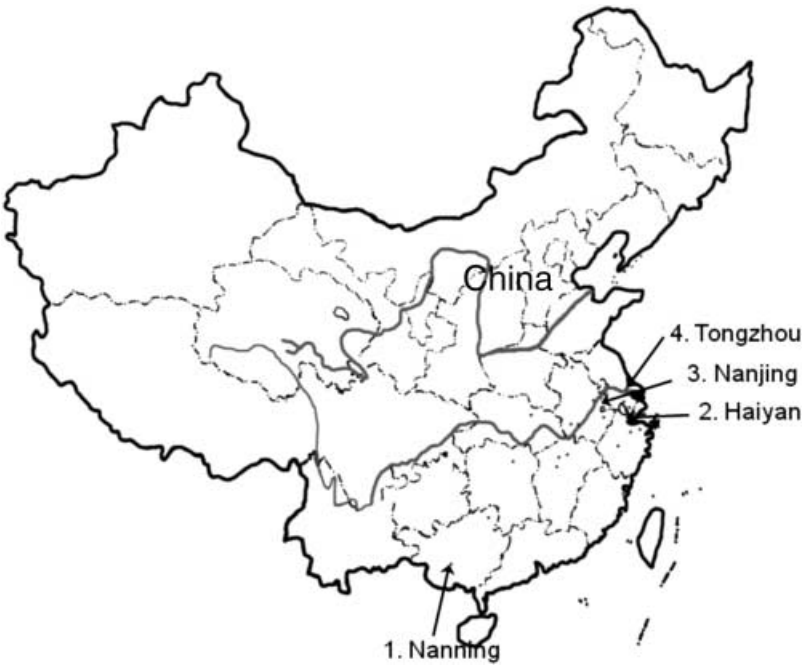

Figure 1 Map showing four locations in three provinces for collection of the brown planthopper, *Nilaparvata lugens*, from 2005 to 2007. 1, Nanning, Guangxi; 2, Haiyan, Zhejiang; 3, Nanjing, Jiangsu; and 4, Tongzhou, Jiangsu.

Local and temporal variation in imidacloprid susceptibility

To examine imidacloprid-resistance levels in different rice-growing areas, four populations of *N. lugens* were collected in August from 2005 to 2007 from Nanning (22°7′N, 108°3′E, Guangxi), Haiyan (30°5′N, 120°8′E, Zhejiang), Nanjing (32°0′N, 118°5′E, Jiangsu), and Tongzhou (32°1′N, 121°1′E, Jiangsu) in China (Figure 1). These locations were chosen based on the migration route of *N. lugens* from the southern into the northeastern part of China, the importance of rice production, and history of the insecticide applications in the areas. The field populations were collected from the same farms each year. Approximately 800 adults and 500–600 nymphs were collected from each site and were transferred to the greenhouse on the campus of Nanjing Agricultural University. The insecticide-free hybrid rice (Shanyou 63) at tillering to booting stage was used for maintaining insect colonies and subsequent bioassays. The field-collected insects were mass mated, and the third instars of F_1 progenies were used for bioassays. All insect colonies were maintained at 27 ± 1 °C and L16:D8.

The bioassays were conducted by using the rice stem-dipping method (Zhuang et al., 1999) to examine dose-response of various populations to imidacloprid and other insecticides. Rice seedlings at tillering to booting stage were pulled out and washed thoroughly. Rice stems (about 10 cm in 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. After the rice stems were air-dried for approximately 1 h, moistened cotton was used to wrap the rice roots. The treated rice stems were then placed in a 500-ml plastic cup. Twenty third instars of *N. lugens* were introduced into each plastic cup using a vacuum device. Each bioassay included 5–6 different concentrations of each chemical plus a distilled water-only control, and three plastic cups were arranged as replicates for each concentration. The treated insects were maintained at 27 ± 1 °C and L16:D8. Mortality was recorded after 96 h, except for the buprofezin treatment, which was recorded after 120 h due to its relatively slow action. The nymphs were considered dead if they failed to show movement after being gently prodded with a fine brush.

Resistance selection and resistance stability

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

Laboratory population (Lab). A population of the second generation in 1993 was collected from a rice field near Nanjing (Jiangsu, China). The population was treated once every two generations with imidacloprid using a rice

seedling spray method (Wang et al., 1988) in the laboratory. After 2005, the Lab population was used as a starting population for imidacloprid resistance selection.

Tongzhou population (TZ2006). A population of the third generation in 2006 was collected from a rice field in Tongzhou (Jiangsu, China). This population was reared on insecticide-free rice and then used for evaluating susceptibility change in the population. The imidacloprid-resistant strain (R) was developed in laboratory from the Lab population after being selected with imidacloprid for an additional 23 generations.

The same rice stem-dipping method described above was adopted for resistance selection of the Lab population. Approximately 1000 third instars of every generation were treated with imidacloprid by the rice stem-dipping method and subsequently maintained at 27 ± 1 °C and L16:D8 for 4 days. Survivors were transferred to another cage containing fresh rice seedlings. The mortality for resistance selection was controlled to range between 40 and 70% in order to ensure sufficient survivors to develop and produce enough progeny for the subsequent insecticide selection (the treated concentration was similar to the LC_{50} value of imidacloprid against each generation of *N. lugens*).

To assess resistance stability to imidacloprid in *N. lugens*, the sixth generation of the Lab and TZ2006 populations (after being selected with imidacloprid) was reared on insecticide-free hybrid rice (Shanyou 63) for 17 and 16 generations, respectively, in the laboratory and LC_{50} values were determined every generation. To test resistance changes, resistance selection with imidacloprid was resumed with a part of the eighth generation of the TZ2006 population and LC_{50} values were determined every subsequent generation.

Cross-resistance

The same rice stem-dipping method (Zhuang et al., 1999) was adopted to determine the toxicity of the neonicotinoids, buprofezin, and fipronil against the imidacloprid-resistant strain (R) and the Lab population. Cross-resistance in *N. lugans* was evaluated by examining a cross-resistance ratio for each compound of interest, which was calculated by dividing the LC_{50} of the imidacloprid-resistant R Lab strain by the LC_{50} imidacloprid reference Lab strain.

Data analysis

Probit models of the Polo program (LeOra Software, 1997) were used to estimate parameters of dose-mortality regression for each rice stem-dipping bioassay. Mortality was corrected using Abbott's formula for each probit analysis (Abbott, 1925). The resistance ratio was calculated by dividing the LC_{50} value of a field population by the LC_{50} value of the susceptible strain, unless otherwise stated. Two LC_{50} values were considered to be significantly different if no overlap was found between their 95% confidence intervals. Mean tests were conducted using Student's t-test ($P<0.05$).

Results

Local and temporal variation of imidacloprid susceptibility

Data from the 3-year resistance monitoring study (2005–2007) revealed variable resistance ratios in the four populations (Figure 2). Among the four populations sampled in 2005, the Tongzhou population obtained the highest resistance ratio (RR = 799), which was almost four times higher than the Nanning population, with the lowest resistance ratio of 200-fold. The other two populations (Nanjing and Haiyan) had resistance ratios of 558- and

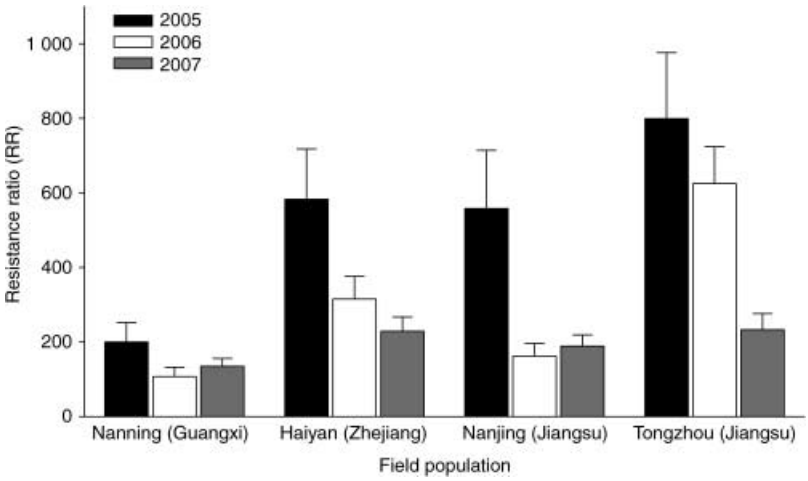

Figure 2 Differential resistance levels to imidacloprid in four populations of *Nilaparvata lugens*, surveyed in 2005, 2006, and 2007, compared with the susceptible strain. The error bars represent the 95% confidence intervals of the resistance ratios.

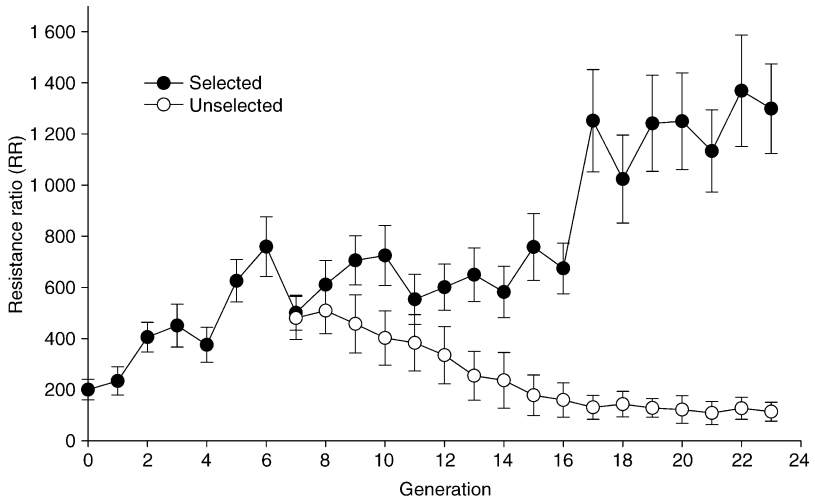

Figure 3 Changes of imidacloprid resistance in the laboratory population (Lab) caused by different imidacloprid selection pressures, continuing selection, and cessation of selection. The error bars represent the 95% confidence intervals of the resistance ratios.

583-fold, respectively. In 2006, resistance ratios decreased in all four populations with the Nanjing population showing the largest (more than three times) drop compared to the previous year level. The Tongzhou population still maintained the highest resistance ratio, which was up to six times higher than the Nanning population. Local variations in 2007 decreased to a relatively lower level than those detected in the two previous years.

Resistance stability and changes

The Lab population of *N. lugens* was selected continuously (every generation) with imidacloprid for 23 generations in the laboratory (Figure 3). Resistance to imidacloprid in the Lab strain increased by 6.5-fold over the 23-generation selection period, that is, from 200-fold in the starting generation to 1 298-fold in the 23rd generation. The results also indicated that the resistance ratio may not yet have approached a plateau or maximum, even though the insect had already developed 1 298-fold resistance to imidacloprid. Continuing selection may further increase resistance in *N. lugens*. Meanwhile, to assess the stability of imidacloprid resistance in *N. lugens*, approximately 1 000 third-instar nymphs of the sixth generation were used for a selection interruption test. A sharp decrease in resistance was detected immediately after removal of the selection pressure (Figure 3). After 17 generations without imidacloprid selection, the resistance ratio descended from 759- to 114-fold. During the last five generations tested, the resistance ratio fluctuated around 120-fold, suggesting that a slow or incomplete recovery of the susceptibility was expected once the population of *N. lugens* had developed resistance to the chemical.

Another experiment involved the Tongzhou population (TZ2006) for testing resistance stability. TZ2006 had developed 625-fold resistance to imidacloprid in the field. After being maintained on insecticide-free rice for 16 generations, resistance dropped to 105-fold (Figure 4). The first eight generations after selection was stopped showed the fastest rate of resistance loss. The resistance ratio of the ninth generation reached 122-fold, and only a slight change in resistance (RR = 105–129-fold) occurred in the subsequent seven generations, suggesting that the population also reached a sustainable resistance level similar to that of the Lab strain. In addition, a portion of the eighth generation of the TZ2006 population was used to study resuming selection with imidacloprid. The resistance ratio in the population quickly increased from 155- to 678-fold after each of the eight subsequent generations were treated with imidacloprid, a figure very similar to the original resistance level (Figure 4).

Cross-resistance to other insecticides

Cross-resistance to eight other insecticides was determined by comparing the resistance ratio of a highly imidacloprid-resistant strain (R: selected from Lab with RR = 1 298-fold) with the resistance ratio of a relatively low imidacloprid-resistant population (Lab: RR = 200-fold). The resistant strain (R) showed substantial cross-resistance to imidaclothiz ($t = 2.96$, d.f. = 1, $P<0.05$), thiacloprid ($t = 2.98$, d.f. = 1, $P<0.05$), and acetamiprid ($t = 1.73$, d.f. = 1, $P<0.05$), and slight levels of cross-resistance to dinotefuran ($t = 1.19$, d.f. = 1, $P>0.05$) and thiamethoxam ($t = 0.35$, d.f. = 1, $P>0.05$). There was no cross-resistance to nitenpyram ($t = 0.43$, d.f. = 1, $P>0.05$), buprofezin($t = 0.6$, d.f. = 1, $P>0.05$), and fipronil ($t = 0$, d.f. = , $P>0.05$) (Table 1).

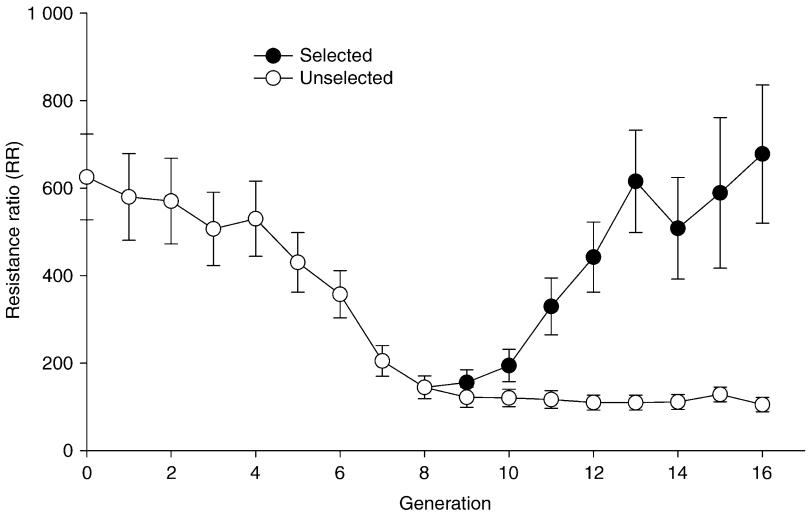

Figure 4 Changes of imidacloprid resistance in the Tongzhou population (TZ2006) influenced by different imidacloprid selection pressures, discontinuing selection, and resuming selection. The error bars represent the 95% confidence intervals of the resistance ratios.

Discussion

Imidacloprid resistance might be a major factor contributing to the frequent outbreaks of *N. lugens* in recent years (Cheng et al., 2003; Cheng & Zhu, 2006; Gao et al., 2006; Wang & Wang, 2007). *Nilaparvata lugens* is a highly migratory insect and is able to travel long distances between the southern and the northeastern part of China (Cheng et al., 1979). Resistance development to insecticides in *N. lugens* was expected to be slow, because it was thought that resistance could be diluted in the process of migration (Zhuang et al., 2004).

However, imidacloprid has been an important chemical for controlling *N. lugens* in China from the early 1990s to 2005. The chemical was used to control *N. lugens* not only in the emigrating region but also in the immigrating region (Liu et al., 2003). Farmers applied imidacloprid to every generation of *N. lugens* to prevent its outbreak in many rice growing areas. Compared to imidacloprid, other insecticides were seldom used because of their relatively lower efficacy against *N. lugens*. The widespread and intensive use of imidacloprid has also been observed in other Asian countries (Wang & Wang, 2007). In addition, imidacloprid, being a systemic insecticide, exhibited prolonged residual activity, which is likely to generate persistent selection pressure for resistance development in *N. lugens* (Liu et al., 2003). Our laboratory selection demonstrated that *N. lugens* was able to achieve a very high level of resistance to imidacloprid under constant selection pressure. Further resistance surveys in 2005 indicated that four field populations of *N. lugens* developed very high resistance levels to imidacloprid (RR = 200–799-fold), and its efficacy against the insect decreased significantly in the Yangtze River Delta areas, including Jiangsu, Zhejiang, Anhui, and other provinces in the same year (CY Diao, unpubl.). Therefore, widespread and intensive use of imidacloprid could be a major driving force for accelerating resistance development in *N. lugens*. Our 3-year resistance monitoring showed that all four populations had developed very high resistance levels to imidacloprid. Resistance development to certain insecticides due to their long application history can also be seen in other insects (Denholm et al., 1998; Mohan & Gujar, 2003; Sayyed et al., 2005; Zhao et al., 2006; Yu & McCord, 2007).

Because imidacloprid has been almost the only insecticide used for controlling *N. lugens* in most of the rice-growing area, migration no longer postpones resistance development substantially (Cheng & Zhu, 2006). The results from resistance monitoring indicated that resistance to imidacloprid in *N. lugens* substantially increased following the immigration of *N. lugens* from the southern into the northeastern part of China, that is, the resistance ratio increased from 200-fold in Nanning to 583-fold in Haiyan, and from 558-fold in Nanjing to 799-fold in the Tongzhou population in 2005. Similarly in 2006 and 2007, the resistance levels of the four populations fluctuated along the migration route following the same pattern as found in 2005. This phenomenon was closely associated with the widespread use of imidacloprid in southern China and other southern Asian countries. The northeastern-bound migration subsequently allowed highly resistant insects to move to the northeastern rice growing areas, where they then were subjected to further selection. Therefore, resistance to imidacloprid in *N. lugens* increased dramatically in the northeastern rice areas after migration.

Table 1 Cross-resistance to various neonicotinoid insecticides and to buprofezin and finopril in the imidacloprid-resistant strain of *Nilaparvata lugens*

Insecticide	Population	Slope (SE)	LC_{50} (95% CI) mg a.i./l	χ^2 (d.f.)	Resistance ratio[1]	Cross–resistance ratio[2]
	S[3]	2.15 (0.12)	0.08 (0.05–0.11)	1.7 (3)	1.0	
Imidacloprid	Lab	1.97 (0.18)	16.01 (13.36–19.39)	0.9 (2)	200.1	
	R	2.02 (0.16)	103.88 (86.07–128.79)	0.3 (3)	1 298.5	6.5
	S	2.09 (0.20)	0.33 (0.27–0.40)	0.7 (4)	1.0	
Imidaclothiz	Lab	2.47 (0.19)	15.22 (13.03–17.88)	2.6 (4)	46.1	
	R	3.04 (0.23)	27.09 (23.45–31.58)	2.9 (3)	82.1	1.8
	S	1.35 (0.11)	13.50 (10.60–17.70)	1.2 (3)	1.0	
Thiacloprid	Lab	2.57 (0.21)	25.27 (21.52–29.51)	4.5 (3)	1.9	
	R	3.09 (0.31)	46.48 (40.44–53.73)	4.7 (4)	3.4	1.8
	S	2.46 (0.22)	7.55 (6.42–9.01)	1.0 (1)	1.0	
Acetamiprid	Lab	2.21 (0.17)	8.27 (6.55–9.98)	2.6 (4)	1.1	
	R	2.13 (0.13)	12.86 (10.83–15.26)	3.2 (3)	1.7	1.6
	S	2.71 (0.25)	0.14 (0.11–0.18)	0.7 (1)	1.0	
Dinotefuran	Lab	2.31 (0.18)	0.19 (0.16–0.23)	4.1 (4)	1.4	
	R	2.48 (0.22)	0.27 (0.21–0.37)	2.8 (7)	1.9	1.4
	S	2.18 (0.16)	0.11 (0.088–0.12)	1.2 (2)	1.0	
Thiamethoxam	Lab	2.03 (0.19)	0.69 (0.58–0.83)	3.7 (4)	6.3	
	R	1.43 (0.17)	0.78 (0.55–1.14)	3.1 (4)	7.1	1.1
	S	2.17 (0.21)	0.47 (0.25–0.61)	0.9 (2)	1.0	
Nitenpyram	Lab	4.60 (0.36)	0.65 (0.58–0.73)	2.6 (4)	1.4	
	R	3.57 (0.23)	0.70 (0.61–0.79)	1.9 (2)	1.5	1.1
	S	1.25 (0.18)	0.066 (0.058–0.074)	1.7 (3)	1.0	
Buprofezin	Lab	2.65 (0.21)	0.21 (0.18–0.25)	2.4 (4)	3.4	
	R	2.38 (0.30)	0.24 (0.20–0.28)	2.1 (3)	3.6	1.1
	S	2.15 (0.12)	0.039 (0.032–0.046)	2.9 (3)	1.0	
Fipronil	Lab	2.48 (0.15)	0.12 (0.099–0.14)	3.1 (2)	3.1	
	R	2.84 (0.20)	0.12 (0.10–0.14)	2.9 (4)	3.1	1.0

[1]Resistance ratio: LC_{50} value of R or Lab/LC_{50} value of S.
[2]Cross-resistance ratio: LC_{50} value of R/LC_{50} value of Lab.
[3]S, susceptible strain to provide baseline to imidacloprid.

Compared to the resistance levels in 2005, the resistance ratios in all four populations dropped slightly in 2006 and 2007. Decreased imidacloprid use in 2006 and 2007 may have contributed to the decrease in resistance in *N. lugens*. In 2005, the regulation authority partially suspended imidacloprid use for *N. lugens* control in China. Buprofezin, fipronil, chlorpyrifos, isoprocarb, and dichlorvos were recommended as alternatives and as candidates for rotation. Therefore, the imidacloprid resistance level in *N. lugens* appears to have decreased due to the reduced selection by imidacloprid. This phenomenon suggests that rotating imidacloprid with other insecticides possessing no cross-resistance is an important practice for susceptibility recovery and for delaying resistance development to imidacloprid in *N. lugens*.

It is well known that resistance development in insects is the consequence of insecticide selection (Wang et al., 2005; Amorim et al., 2007). Once the selection pressure is removed, resistance development will stop or even decline (Tabashnik et al., 1994; Wu et al., 1996). In this study, we observed that the resistance ratio of two populations decreased as expected when resistance selection was stopped. We further discovered that imidacloprid resistance in *N. lugens* rebounded immediately after the resumption of insecticide selection.

Once an *N. lugens* population has obtained a high level of resistance to imidacloprid, it might be able to maintain a sustainable level of resistance after removal of selection pressure for several generations. In this study, we found that resistance levels in both Tongzhou and in the Lab populations tended to decrease after the removal of selection pressure, and then stabilized at similar levels, that is, RR = 105–129-fold, for eight and five generations, respectively. However, complete recovery of susceptibility

was not seen, suggesting that a sustainable level of resistance was reached in the populations without insecticide selection. Similar situations have also been observed in other insects (Georghiou, 1964; Bauernfeind & Chapman, 1985; Zhao et al., 1993; Wu et al., 1996; Qu et al., 2005). In the field, resistance levels in *N. lugens* fluctuated, closely corresponding to the intensity of imidacloprid applications. The results of our 3-year field surveys were consistent with changes in resistance detected from laboratory selections.

Under laboratory conditions, the rate of resistance recession after removal of the selection pressure depended on genotypic composition and relative fitness of resistance genes in the colony (Georghiou, 1964, 1972). Although *N. lugens* was able to develop a high level of resistance to imidacloprid in the laboratory, resistance was not stable and declined after selection stopped. This phenomenon might be associated with an incompletely recessive trait of imidacloprid resistance controlled by multiple genes and significant fecundity and egg hatch costs (Zhao et al., 2000; Liu & Han, 2006; Baker et al., 2007). Achieving a homozygous colony was slow because a relatively low dose was used for the laboratory selection. After imidacloprid selection was stopped, heterozygous and homozygous susceptible individuals could quickly increase in frequency in the colony due to their fitness advantages over resistant individuals (Liu & Han, 2006; Baker et al., 2007). This phenomenon is consistent with the hypothesis that imidacloprid resistance is controlled by multiple incompletely recessive genes that have a fitness cost. Using alternative insecticides without cross-resistance to imidacloprid for *N. lugens* control could have relieved selection pressure on the target insect. Therefore, the resistance levels to imidacloprid declined in 2006 and 2007 in all four populations. On the other hand, alternating and rotating imidacloprid with other insecticides without cross-resistance may minimize or slow resistance development in the insect.

In addition to the direct examination of resistance development to imidacloprid in the target insect, risk assessment of cross-resistance is another important part of resistance management. Because cross-resistance might be involved in both functionally similar and dissimilar chemicals (Shen & Wu, 1995; Zhao et al., 1995; Elzen, 1997), assessment of cross-resistance must include not only neonicotinoid insecticides, but also non-neonicotinoids, which have the potential to be alternated or rotated with imidacloprid to control resistant populations of *N. lugens*. In this study, we detected substantial cross-resistance to imidaclothiz, thiacloprid, and acetamiprid in a highly imidacloprid-resistant strain of *N. lugens*. The insects also showed slight levels of cross-resistance to dinotefuran and thiamethoxam, but not to nitenpyram. In addition, the highly imidacloprid-resistant strain of *N. lugens* showed no cross-resistance to buprofezin (an insect growth regulator) and fipronil (a phenylpyazole insecticide). Therefore, to relieve imidacloprid selection pressure and to recover imidacloprid susceptibility in *N. lugens*, we suggest that nitenpyram, buprofezin, and fipronil can be used in resistance management programs for alternation and rotation with imidacloprid.

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