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Current status of insecticide resistance in rice planthoppers in Asia

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In 2003, the development of insecticide resistance against neonicotinoids (mainly imidacloprid) in the brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), was first observed in Thailand and has since been found in other Asian countries such as Vietnam, China, and Japan. However, the LD_{50}-values of BPH and the whitebacked planthopper (WBPH), *Sogatella furcifera* (Horváth), against both neonicotinoid and phenylpyrazole insecticides have been poorly reported in many Asian countries. We thus determined and compared the insecticide susceptibility (topical LD_{50}s) of BPH and WBPH that were collected in Japan from 2005 to 2007 and from East and Southeast Asian countries in 2006. Species-specific changes in insecticide susceptibility were found in Asian rice planthoppers (i.e., BPH for imidacloprid and WBPH for fipronil). The topical LD_{50}-values for imidacloprid in the BPH populations collected from East Asia (Japan, China, Taiwan) and Vietnam in 2006 were significantly higher than in those collected from the Philippines, suggesting that insecticide resistance in BPH against imidacloprid occurred in East Asia and Indochina, but not in the Philippines. The BPH populations indicated a positive cross-resistance between imidacloprid and thiamethoxam. Almost all the WBPH populations from Japan, Taiwan, China, Vietnam, and the Philippines had extremely large LD_{50}-values for fipronil, suggesting that insecticide resistance in WBPH against fipronil occurred widely in East and Southeast Asia.

Keywords: *Nilaparvata lugens*, *Sogatella furcifera*, topical application, imidaclopirid, fipronil

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), and the whitebacked planthopper (WBPH), *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae), are two serious pests of rice throughout Asia. The northern limit of breeding area for these species is around the Red River Delta of Vietnam, where rice (*Oryza sativa*, their only host plant) is cultivated year-round. Neither of these species is able to overwinter successfully in temperate areas (Japan, Korea, and most areas of China), and

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colonization occurs annually following long-distance migration from overwintering area (Kisimoto 1976).

To control these planthoppers, neonicotinoid and phenylpyrazole insecticides such as imidacloprid and fipronil have been used since the mid-1990s in many East Asian countries and Indochina. Treatment methods of these insecticides vary among countries. In Japan, imidacloprid and fipronil are used exclusively for seedling-box treatment to control rice planthoppers. In Vietnam and China, in contrast, these insecticides are usually sprayed on rice fields. In any event, the population densities of BPH and WBPH had been relatively low since the mid-1990s when these insecticides began to be used.

In 2003, however, the development of insecticide resistance against neonicotinoids (mainly imidacloprid) in BPH was first observed in Thailand and has since been found in other neighboring countries such as Vietnam, India, and China (Harris 2006). However, until now, the LD_{50}-values of BPH and WBPH against both neonicotinoid and phenylpyrazole insecticides tested by highly accurate methods such as the topical application method (Fukuda and Nagata 1969) have been poorly reported in many Asian countries. Therefore, we determined and compared the insecticide susceptibility of BPH and WBPH that were collected (1) in Japan from 2005 to 2007 and (2) from East and Southeast Asian countries in 2006.

Insecticide susceptibility of BPH and WBPH immigrating into Japan

Immigrant adults of BPH and WBPH were collected in early July from 2005 to 2007 in Kumamoto (in 2005 and 2007) and Kagoshima (in 2006), Japan. The collected populations were derived from more than 50 pairs of adults. These populations were maintained in the laboratory for 2–5 generations prior to the test using rice seedlings (var. Reihou) at a daylength of 16 h and a temperature of 25 °C.

The insecticide susceptibility of these populations was monitored by a standard topical application method (Fukuda and Nagata 1969). Altogether, ten insecticides (malathion, fenitrothion, MIPC, BPMC, carbaryl, etofenprox, imidacloprid, fipronil, dinotefuran, and thiamethoxam) were tested.

The long-winged female adults within 7 days after emergence were anaesthetized with carbon dioxide for about 5 s prior to treatment. A 0.08 μL droplet of acetone solution was applied topically on the dorsal surface of the thorax with a hand microapplicator (Burkard Manufacturing Company Ltd.). The treated insects were kept at a daylength of 16 h and a temperature of 25 °C, with rice seedlings in a transparent plastic box (5 cm in diameter, 10 cm high). Mortality was determined 24 hours after treatment for all insecticides. All the tests were conducted on 2–5 generations after collection. More than 45 females were used for each concentration. Tests were carried out on 5–6 concentrations. The LD_{50}-value, 95% confidence interval, and slope of regression line were calculated by Bliss's probit method (Bliss 1935). Control mortality was corrected for by using Abbott's formula for each probit analysis. A chi-square test was used to test for heterogeneity ($P = 0.05$).

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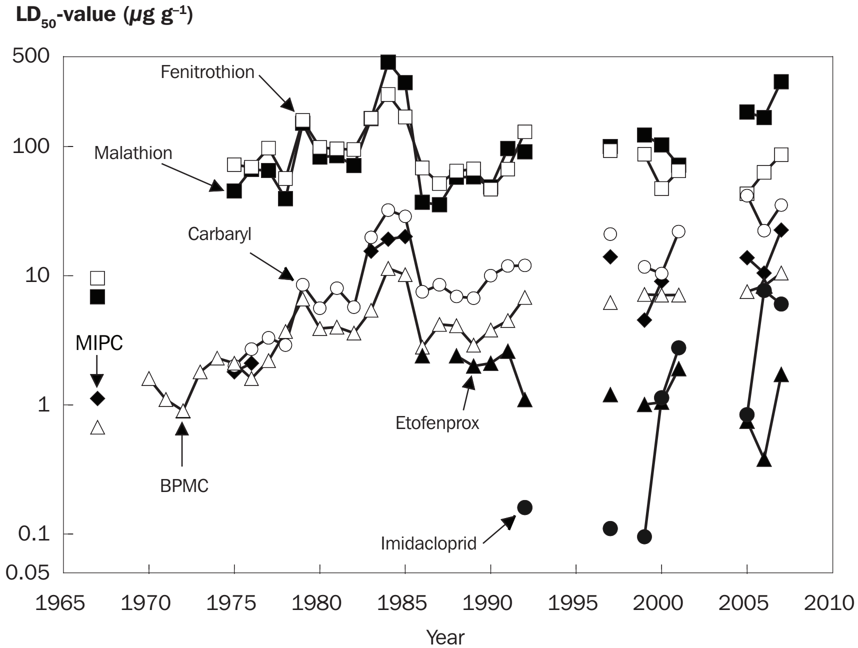

Fig. 1. Annual changes in topical LD_{50}-values of *Nilaparvata lugens* collected in Japan. (1967: Fukuda and Nagata 1969; 1970-91: Hosoda 1983 and unpublished; 1992: Endo and Tsurumachi 2001; 1997: Ping et al 2001; 1999-2000: Nagata et al 2002; 2001: Nagata and Kamimuro 2002; 2005-07: Matsumura et al unpublished.)

The LD_{50}-values for the BPH and WBPH strains collected from 2005 to 2007 in Japan for the seven insecticides (malathion, fenitrothion, MIPC, BPMC, carbaryl, etofenprox, and imidacloprid) were compared with those obtained before 2001 in Japan (Figs. 1 and 2). In general, no significant changes were observed except an increase in LD_{50}-values for imidacloprid in BPH from 1990 to 2007 (Fig. 1). The LD_{50}-value for imidacloprid for the 2000 population was 10 times larger than the 1999 population (Nagata et al 2002), and this trend continued up to 2005. From 2006, a significant increase in LD_{50}-value was observed again for imidacloprid. In contrast to BPH, the LD_{50}-values in WBPH were still low until 2007 (Fig. 2).

Although no previous baseline data have been reported for dinotefuran, fipronil, and thiamethoxam against BPH and WBPH, the LD_{50}-values in WBPH against fipronil and those in BPH against thiamethoxam increased about tenfold during 2005 to 2007, indicating that the insecticide susceptibility has been decreasing (Fig. 3). Dinotefuran is the same neonicotinoid group as imidacloprid, but the LD_{50}-values in BPH in 2005 to 2007 were lower than those against imidacloprid, indicating no cross-resistance between imidacloprid and dinotefuran (Fig. 3).

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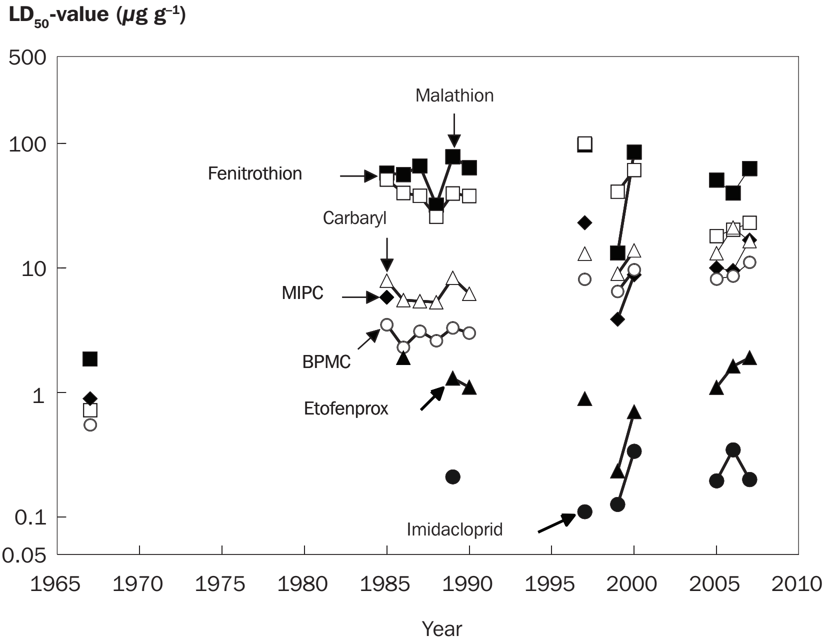

Fig. 2. Annual changes in topical LD_{50}-values of *Sogatella furcifera* collected in Japan. (1967: Fukuda and Nagata 1969; 1985-90: Hosoda 1989, and unpublished; 1997: Ping et al 2001; 1999-2000: Nagata et al 2002; 2005-07: Matsumura et al unpublished.)

Insecticide susceptibility in East and Southeast Asia

The 16 and 17 populations for BPH and WBPH, respectively, were collected from East Asia (Japan, China, Taiwan), Indochina (Vietnam), and Southeast Asia (Philippines) in 2006. The collected populations were derived from more than 50 pairs of adults except for three Philippine populations in 2006. These populations were maintained in the laboratory for 2–5 generations prior to the test using rice seedlings (var. Reihou) at a daylength of 16 h and a temperature of 25 °C. The insecticide susceptibility of these populations was monitored by a standard topical application method (Fukuda and Nagata 1969). Four insecticides (imidaclopirid, thiamethoxam, fipronil, and BPMC) were tested.

In the case of imidaclopirid, the LD_{50}-values for the BPH populations collected from East Asia (Japan, China, Taiwan) and Vietnam were 4.3–24.2 μg g^{-1} and were remarkably larger than for populations collected from the Philippines (0.18–0.35 μg g^{-1}) (P<0.01, Mann-Whitney U test) (Fig. 4). The East Asian and Vietnamese populations had significantly larger LD_{50}-values (0.27–2.16 μg g^{-1}) for thiamethoxam than the Philippine ones (0.41–0.62) (P<0.05, Mann-Whitney U test). There was a significant

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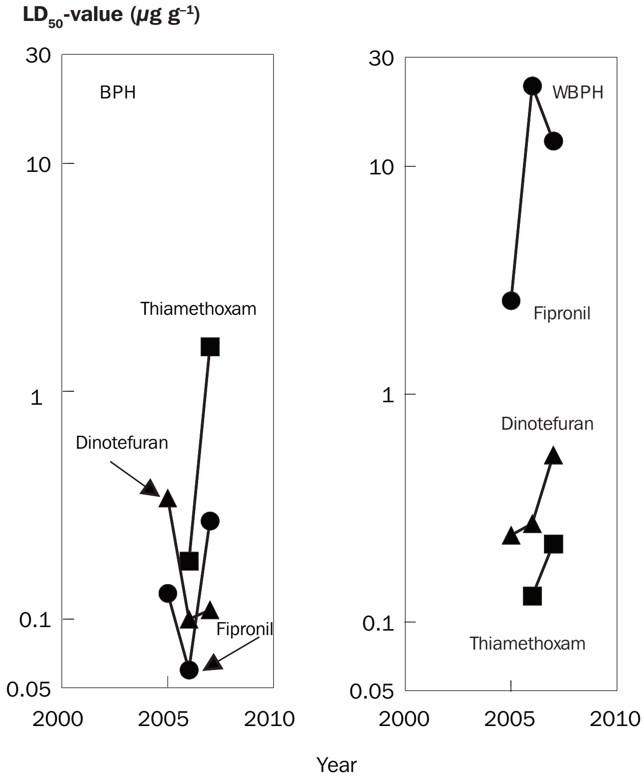

Fig. 3. LD_{50}-values of *Nilaparvata lugens* (left) and *Sogatella furcifera* (right) collected in 2005-07 in Japan (Matsumura et al, unpublished.)

positive relationship between the LD_{50}-values of imidacloprid and thiamethoxam in all the Asian populations (r = 0.72, $P<0.01$) (Fig. 5).

In contrast to the two neonicotinoids, all the Asian BPH populations had much smaller LD_{50}-values (0.06–0.65 μg g^{-1}) for fipronil and no difference was found among locations ($P>0.05$, Mann-Whitney U test) (Fig. 4). In BPMC, the LD_{50}-values were larger (>30 μg/g) in several Vietnamese and Philippine populations than those in other populations, but there was no significant difference among countries ($P>0.05$, Mann-Whitney U test) (Fig. 4).

In WBPH, almost all the populations collected from Japan, Taiwan, China, Vietnam, and the Philippines had much larger LD_{50}-values (19.7–239 μg g^{-1} or more for 24 h after treatment) for fipronil except for several populations from the Philippines (0.3–5.9 μg g^{-1}) and China (3.0 μg g^{-1}) (Fig. 6).

In the case of imidaclopirid, all the WBPH populations had small LD_{50}-values (0.11–0.34 μg g^{-1}). In the case of BPMC, the LD_{50}-values for WBPH ranged from 6.1 to 26.6 μg g^{-1}. There were no significant differences in LD_{50}-values for all three

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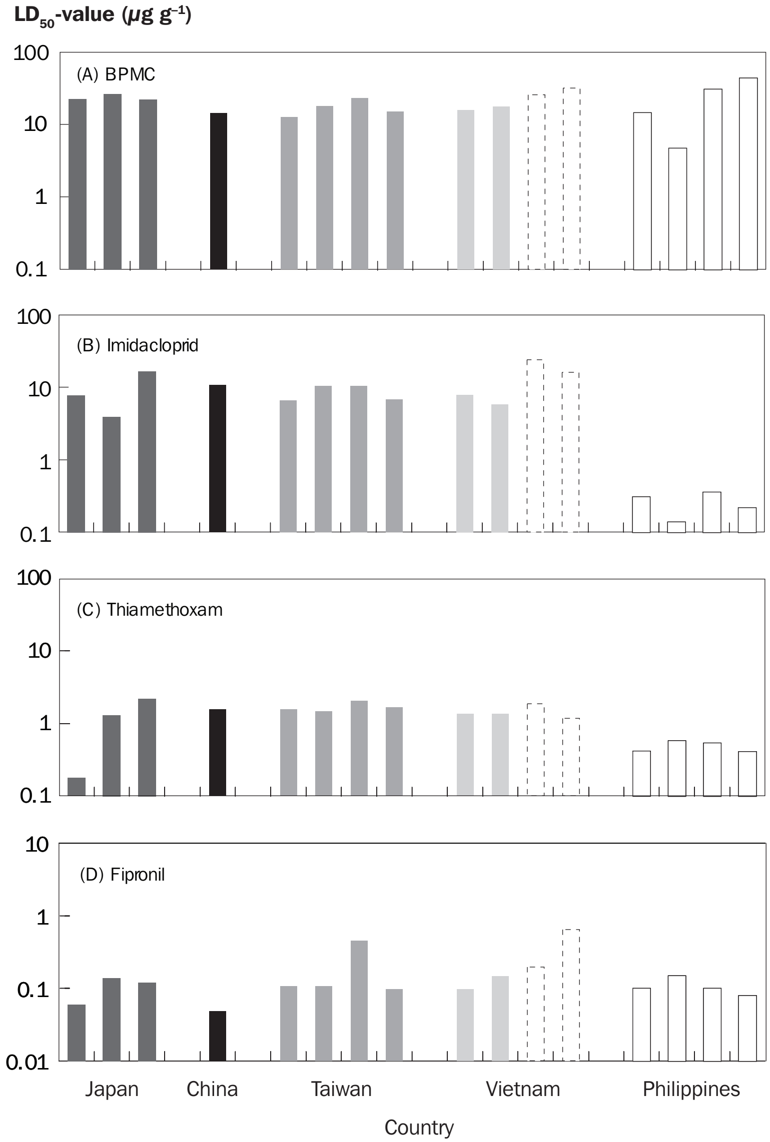

Fig. 4. LD_{50}-values of *Nilaparvata lugens* strains collected in East and Southeast Asia in 2006 (Matsumura et al 2008).

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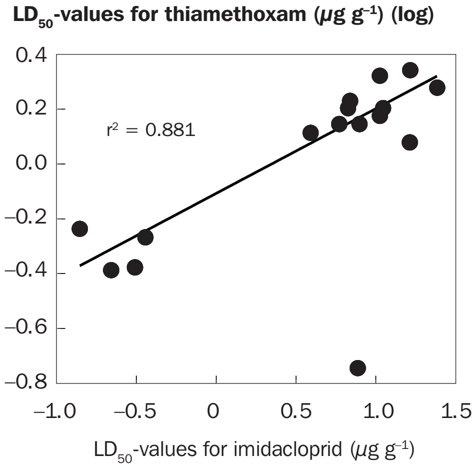

Fig. 5. Relationship between LD_{50}-values for imidacloprid and thiamethoxam in *Nilaparvata lugens* collected in Asia in 2006 (Matsumura et al 2008).

insecticides between East Asian and Southeast Asian WBPH populations ($P>0.05$, Mann-Whitney U test).

Discussion

Imidacloprid has been used widely to control rice planthoppers since the early 1990s in East Asia and Indochina. The topical LD_{50}-values of imidacloprid for BPH were in the range of 0.09–2 µg g^{-1} from 1992 to 2003 in Vietnam, China, and Japan (Endo and Tsurumachi 2001, Ping et al 2001, Nagata et al 2002, Nagata and Kamimuro 2002, Liu et al 2003a,b). In our study, however, the East Asian and Vietnamese BPH populations in 2006 had remarkably higher LD_{50}-values than those before 2003. In contrast, the BPH populations collected in the Philippines in 2006 had similar LD_{50}-values for imidacloprid vis-à-vis those in the East Asian populations before 2003 (Endo and Tsurumachi 2001, Ping et al 2001, Nagata et al 2002, Nagata and Kamimuro 2002, Liu et al 2003a,b). These results suggest that insecticide resistance against imidacloprid occurred only in East Asia and Indochina but not in the Philippines.

In contrast to BPH, no significant differences in LD_{50}-values for imidacloprid were found among Asian WBPH populations except for one Japanese population. The LD_{50}-values in 2006 were similar to those in Japanese and Chinese populations collected in 1992-2001 (0.02–0.33 µg g^{-1}) (Ping et al 2001, Liu et al 2003b). These results suggest that no insecticide resistance against imidacloprid occurred in WBPH in Asia.

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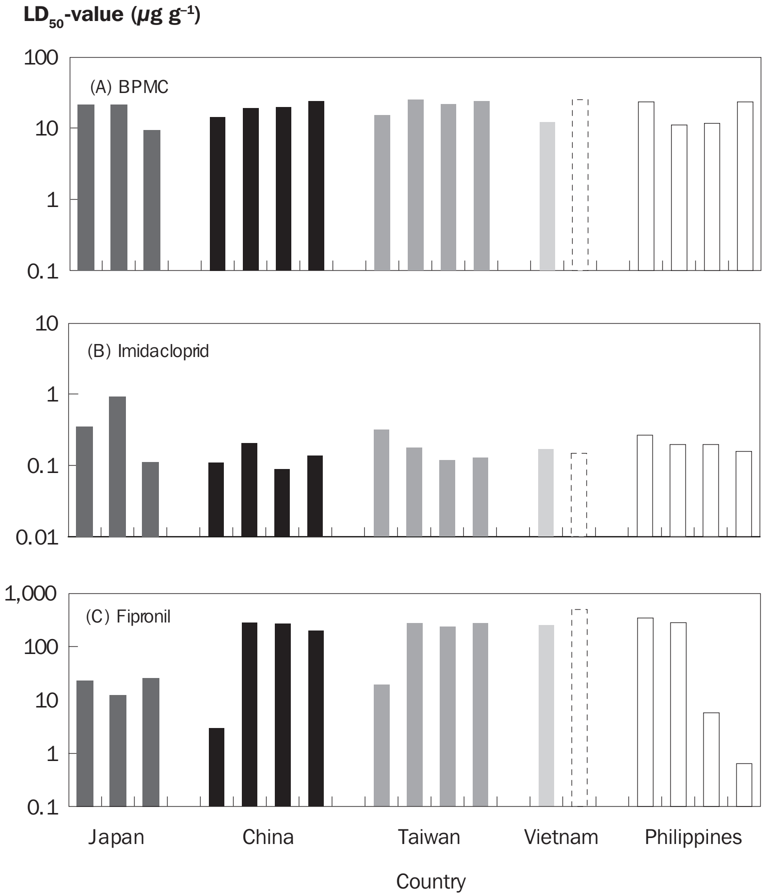

Fig. 6. LD_{50}-values of *Sogatella furcifera* strains collected in East and Southeast Asia in 2006 (Matsumura et al 2008).

Although the LD_{50}-values for thiamethoxam were not so large, the BPH populations indicated a positive cross-resistance between imidacloprid and thiamethoxam. In the case of dinotefuran, another neonicotinoid insecticide, the BPH populations collected in 2005-07 in Japan showed no insecticide resistance. Thus, the cross-resistance with imidacloprid might not occur in all the neonicotinoid insecticides.

Almost all the Asian WBPH populations collected in 2006 had large LD_{50}-values for fipronil at 24 h after treatment. Although no topical LD_{50}-values for fipronil in the field for WBPH populations have been published previously, these results suggest that insecticide resistance of WBPH against fipronil occurred widely in East and Southeast Asian countries.

On the other hand, all the Asian BPH populations had much smaller LD_{50}-values for fipronil, suggesting that no insecticide resistance against fipronil occurred in BPH

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in Asia. However, the LD_{50}-values of these two populations are slightly higher than others. Thus, the monitoring of insecticide susceptibility to fipronil in BPH should be continued in this region.

In the case of BPMC, the LD_{50}-values of BPH and WBPH in 2006 were similar to those in Japan, China, and Vietnam in 1992-2001 (8.8–26 $\mu g\ g^{-1}$ for BPH and 5.1–28 $\mu g\ g^{-1}$ for WBPH) (Endo and Tsurumachi 2001, Ping et al 2001, Nagata et al 2002, Liu et al 2003b). No significant differences were detected among countries.

Our study revealed a species-specific change in insecticide susceptibility in Asian rice planthoppers (i.e., BPH for imidaclopirid and WBPH for fipronil). Imidacloprid has been used commonly to control BPH in the latter stage of rice in Vietnam and China (around May to early June in winter-spring rice cropping in northern Vietnam). Fipronil has been used commonly to control the rice leaffolder, *Cnaphalocrocis medinalis* (Guenée), and rice stem borers in the early stage of rice in Vietnam and China (around early April in winter-spring rice cropping in northern Vietnam). Spraying fipronil in the early season could also be more effective on WBPH than on BPH because WBPH increases earlier than BPH in the rice-growing season. This could be a possible reason insecticide resistance against fipronil occurred only on WBPH species. The overuse of insecticides is often the precursor to the development of insecticide resistance and many Asian countries rely heavily on a limited number of compounds for planthopper control (Nagata et al 2002, Sun et al 1996).

Our study suggests that the insecticide resistance of BPH against imidacloprid did not occur in the Philippines. This is because BPH outbreaks have not occurred recently and imidacloprid has not been used commonly in the Philippines. In contrast, fipronil has been used commonly to control rice stem borers in the Philippines. For this reason, the insecticide susceptibility of WBPH against fipronil in the Philippines was as low as that in East Asia and Vietnam.

The species-specific insecticide resistance against different insecticides might have also occurred by the difference in mode of action of insecticides. Liu et al (2005) found that a nicotinic acetylcholine receptor (nAChR) mutation confers target-site resistance to imidacloprid in BPH. However, this target-site resistance in BPH was selected only in the laboratory and was never found in a field strain. Although the target site of fipronil (GABA receptors) is different from that of imidacloprid (see review of Raymond-Delpech et al 2005), no detailed information is available for the mechanism of resistance to fipronil in WBPH. Further comparative studies on the mode of action of neonicotinoid and phenylpyrazole insecticides against BPH and WBPH are needed to explain the species-specific development of insecticide resistance against neonicotinoid and phenylpyrazole insecticides as well.

In the Mekong Delta of southern Vietnam, outbreaks of the two BPH-transmitted virus diseases, rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV), have occurred since 2005 (Chien et al 2007), resulting in a heavy use of insecticides to control BPH. Our study showed that the LD_{50}-values in two southern Vietnamese BPH populations tended to be higher than those in the other locations for BPMC, imidacloprid, and fipronil. Thus, it is important to continue to carefully monitor the

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status of insecticide susceptibility in BPH against these insecticides in southern Vietnam and neighboring countries such as Thailand.

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