# Current Status of Insecticide Resistance and Virulence to Resistant Rice Varieties in Asian Rice Planthoppers

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## Abstract

Insecticide susceptibility and virulence to resistant rice varieties were determined and evaluated in the brown planthopper (BPH) and the whitebacked planthopper (WBPH), collected from Asian countries (Japan, China, Taiwan, northern Vietnam, southern Vietnam, and Philippines) in 2006. Insecticide susceptibility was determined by a topical application method using four insecticides. Virulence to resistant rice varieties was evaluated using five differential rice varieties carrying different planthopper resistance gene(s). Species-specific changes in insecticide susceptibility were found in Asian rice planthoppers (i.e. imidacloprid resistance in BPH and fipronil resistance in WBPH). The topical $LD_{50}$-values for imidacloprid in the BPH strains in East-Asia (Japan, China, and Taiwan) and Vietnam were significantly higher than those in the Philippines, suggesting that insecticide resistance in BPH against imidacloprid occurred in East Asia and Indochina, but not in the Philippines. Almost all the WBPH populations collected had extremely large $LD_{50}$-values for fipronil, suggesting that insecticide resistance in WBPH against fipronil occurred widely in East and Southeast Asia. The virulence of Asian BPH strains was classified into three groups: (1) The BPH strains in East Asia and northern Vietnam were virulent to Mudgo (*Bph1*) and ASD7 (*bph2*) but avirulent to other three varieties, (2) The BPH strains in Southeast Asia (the Philippines) were virulent to Mudgo, ASD7 and also partially virulent to Babawee (*bph4*), and (3) The BPH strains in southern Vietnam were highly virulent to Babawee in addition to Mudgo and ASD7. The varieties Rathu Heenati (*Bph3*, *Bph17*) and Balamawee (*Bph9*) still have a broad spectrum of resistance against all the Asian BPH strains tested.

**Keywords:** topical application, imidaclopirid, fipronil, virulence, resistant gene

## Introduction

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), and the whitebacked planthopper (WBPH), *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae), are the two serious pests of rice throughout Asia. The northern limit of breeding area for these species is around the Red River Delta, Vietnam, where rice (*Oryza sativa*, their only host plant) is cultivated all year round. Neither of these species is able to overwinter successfully in temperate areas (Japan, Korea, and most areas of China), and 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 middle 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 the rice planthoppers. In Vietnam and China, in contrast, these insecticides are usually sprayed on the rice fields. In any event, the population densities of the BPH and WBPH had been relatively low since middle 1990s when these insecticides were began to use.

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. However, until now the $LD_{50}$-values of BPH and WBPH against both neonicotinoid and phenylpyrazole insecticides tested by highly accurate methods such as topical application method have been poorly reported in many Asian countries. Therefore, the insecticide susceptibility of BPH and WBPH which were collected from East and Southeast Asian countries in 2006 were determined and compared.

Against BPH, many resistant varieties were released since 1973 by the International Rice Research Institute (IRRI) and other countries (Khush, 1979). Wide-scale monoculture of rice varieties with monogenic resistance to the BPH resulted in the development of a new biotype of BPH that broke the resistance of rice to the BPH in Philippines, Indonesia, Vietnam and the Solomon Islands during 1974-1977, soon after the introduction of resistant varieties (Khush, 1979; Sogawa, 1982; Gallagher et al., 1994). Changes in BPH biotypes are a continued threat to increased rice production in Asia.

Monitoring of the virulence of BPH to resistant varieties has been conducted in many Asian countries. The BPH migrate to Japan became virulent to a resistance gene *Bph1* around 1987-1990 (Sogawa, 1992a, b). This trend was also found in China and northern Vietnam in the same period. The BPH populations in Japan became virulent to *bph2* beginning in 1997 (Tanaka, 1999; Tanaka and Matsumura, 2000), and the virulence remained at that high level through 1999. This change also has been occurred coincidentally in China and northern Vietnam. However, the current status of virulence of BPH to resistant rice varieties in Asia is unknown. Thus, current virulent status of the Asian BPH populations to the rice differential varieties was evaluated based on the feeding response.

## Materials and Methods

### Insecticide susceptibility

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. These populations were maintained in the laboratory for 2-5 generations prior to the test using rice seedlings (var. Reihou) at a day length of 16h 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, thiametoxam, fipronil, and BPMC) were tested. Thiamethoxam was tested only for BPH.

The long-winged female adults within 7 days after emergence were anaesthetized with carbon dioxide for about 5s prior to treatment. A 0.08μl 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 day length of 16h and a temperature of 25°C, with rice seedlings in a transparent plastic box (5cm diameter, 10cm high). Mortality was determined 24h after treatment for all insecticides. In case of fipronil on WBPH, mortality was also determined 48 and 72 hours after treatment because mortality was less than 50% at 24h after treatment in some populations. 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 the Bliss's probit method. Control mortality was corrected for by using Abbott's formula for each probit analysis..

**Virulence to resistant rice varieties**

Five rice differential varieties with different resistance gene(s) to BPH: Mudgo (carrying *Bph1*), ASD7 (carrying *bph2*), Rathu Heenati (carrying *Bph3* and *Bph17*), Babawee (carrying *bph4*), Balamawee (carrying *Bph9*) and Taichung 65 (T65) (no resistance gene) were used. The 10 strains of BPH were collected from East Asia (Japan, China, Taiwan), Indochina (Vietnam) and Southeast Asia (Philippines) in 2006. The BPH strains were the same as those used in the test of insecticide susceptibility described above. These strains were maintained in the laboratory prior to the test using susceptible variety (var. Reihou) at a day length of 16h and a temperature of 25°C.

Seeds of the test variety were sown individually in plastic cups (220ml) with soil. One-month old seedlings were trimmed to 15cm height, and each trimmed plant was covered with a transparent plastic cylindrical cage (5cm D x 25cm H). Five brachypterous (short-wing form) females within 24h after emergence were released into the cage and the open end was covered with a nylon cloth. The number of surviving insects and the shape of their abdomens were monitored at 5 days after infestation. We classified females survived for five days and with heavily swollen abdomens as virulent, and females died within five days as avirulent. This system of classification is similar to that used by Tanaka (2000) for identifying virulent and avirulent females. Eight independent replicates of the experiment were performed.

## Results

**Insecticide susceptibility**

In case of imidaclopirid the $LD_{50}$-values for the BPH populations collected from East-Asia (Japan, China, Taiwan) and Vietnam were remarkably larger than those collected from the Philippines (Fig. 1). The East-Asian and Vietnam populations had significantly larger $LD_{50}$-values for thiamethoxam to compare with Philippines ones. In contrast to the two neonicotinoids (imidacloprid and thiamethoxam), all the Asian BPH populations had much smaller $LD_{50}$-values for fipronil and no difference was found among locations. In BPMC, the $LD_{50}$-values were larger in several Vietnam and Philippines populations than those in other populations, but there was no significant difference among countries.

In WBPH, almost all the populations collected from Japan, Taiwan, China, Vietnam and the Philippines had extremely larger $LD_{50}$-values for fipronil except for several populations from the Philippines and China (Fig. 2). In case of imidaclopirid all the WBPH populations had small $LD_{50}$-values. In case of BPMC the $LD_{50}$-values for WBPH ranged from 6.1-26.6 μg/g. There were no significant differences in $LD_{50}$-values for all the three insecticides between East-Asian and Southeast Asian WBPH populations.

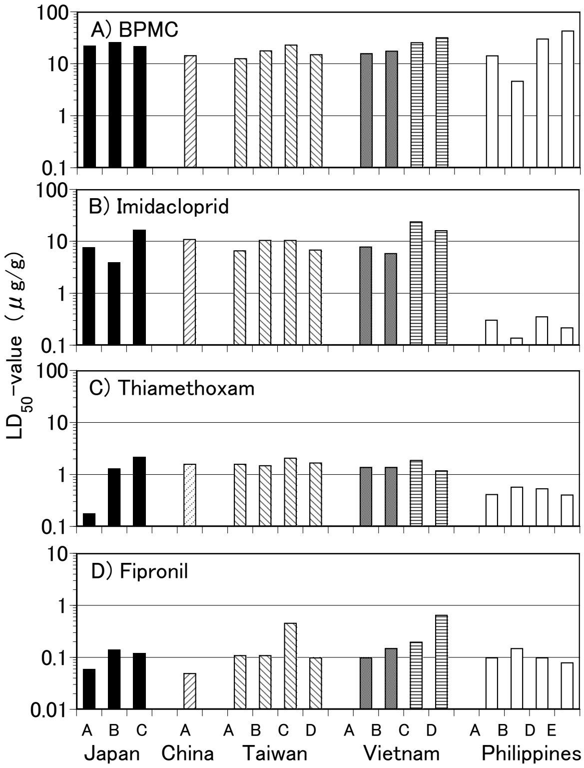


Fig. 1. The $LD_{50}$-values of *Nilaparvata lugens* strains collected in East and Southeast Asia in 2006 (Matsumura et al., 2008).

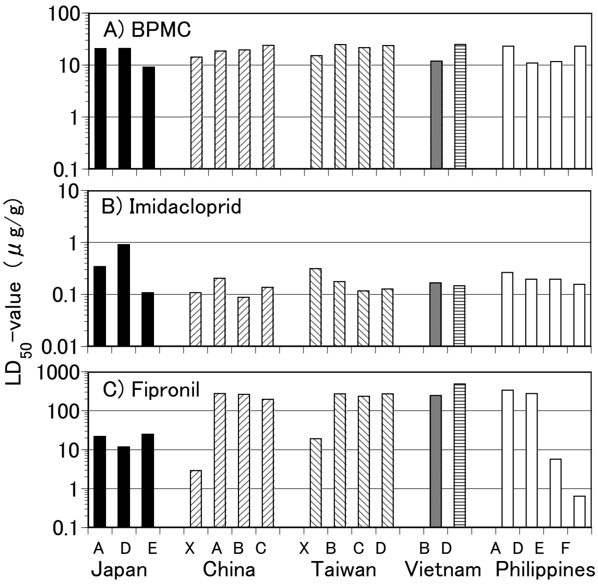


Fig. 2. The $LD_{50}$-values of *Sogatella furcifera* strains collected in East and Southeast Asia in 2006 (Matsumura et al., 2008).

## Virulence to resistant rice varieties

The survival rate of Taiwan, China, Japan, and northern Vietnam BPH females on Mudgo and ASD7 were high and all the surviving females showed a swollen abdomen (Fig. 3). In contrast, only small proportions of these strains survived on other three varieties (Rathu Heenati, Babawee and Balamawee) and all of them did not show the swollen abdomen. The Philippines strains were virulent on Mudgo and ASD7 and showed high survival rates, and high proportions of females with swollen abdomen. Survival rates ranged from 31.4 to 37.1% on Babawee and surviving females became swollen abdomen in all Philippines strains. On the other hand, high proportions of southern Vietnam strains survived on Mudgo, ASD7 and Babawee, and almost all of them show swollen abdomen. In contrast, small proportions of all Asian BPH strains survived on Rathu Heenati and Balamawee, and all of them did not show the swollen abdomen.

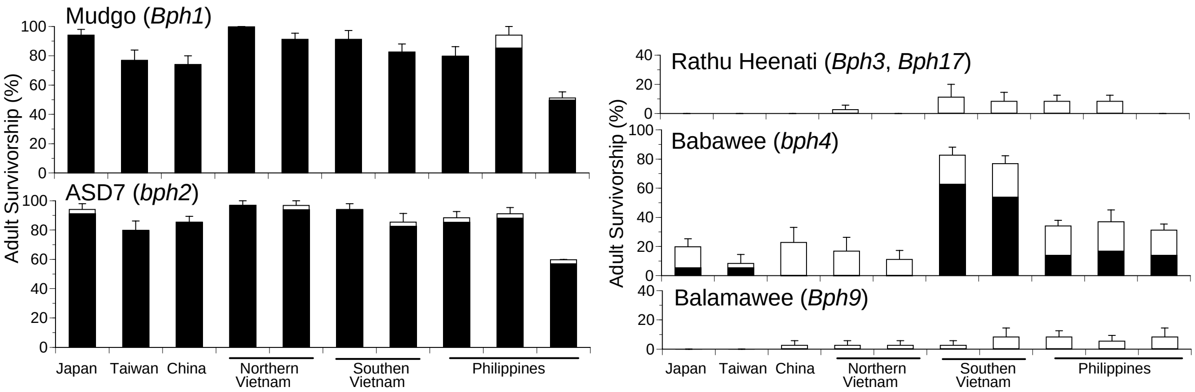


Fig. 3. Adult survivorship (solid bar) and the proportion of females with swollen abdomen (open bar) of *Nilaparvata lugens* strains collected in East and Southeast Asia in 2006 on rice differential varieties carrying the resistance genes (Myint et al., unpublished).

## Discussion

Imidacloprid has been used widely to control rice planthoppers since early 1990s in East-Asia and Indochina. The topical $LD_{50}$-values of imidacloprid for BPH were in range of 0.09-2μg/g from 1992 to 2003 in Vietnam, China, and Japan (Endo et al., 2001; Ping et al., 2001; Nagata et al., 2002; Nagata and Kamimuro, 2002; Liu et al., 2003a, 2003b). In the present study, however, the East-Asian and Vietnam 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 to compare with those in East-Asian population before 2003. 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 with those in Japanese and Chinese populations collected in 1992-2001 (Ping et al., 2001; Liu et al., 2003b). These results suggest that no insecticide resistance against imidacloprid occurred in WBPH in Asia. However, it should continue to monitor the insecticide susceptibility of WBPH against fipronil because one Japanese population had a slightly higher $LD_{50}$-value than others.

Almost all the Asian WBPH populations collected in 2006 had large $LD_{50}$-values for fipronil at 24h after treatment. Although no topical $LD_{50}$-values for fipronil in the field WBPH populations have been published previously, these results suggest that insecticide resistance of WBPH against fipronil occurred widely in East-Asian 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 in Asia. However, the $LD_{50}$-values of these two populations are slightly larger than others. Thus, the monitoring of insecticide susceptibility to fipronil in BPH should be continued in this region.

In case of BPMC, the $LD_{50}$-values of BPH and WBPH in 2006 were similar with those in Japan, China, and Vietnam in 1992-2001 (Endo et al., 2001; Ping et al., 2001; Nagata et al., 2002; Liu et al., 2003b). No significant differences were detected among countries.

The present 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 later 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 the rice stem borers in early stage of rice in Vietnam and China (around early April in winter-spring rice cropping in northern Vietnam). Spraying fipronil in early season could also be more affected on WBPH than on BPH, because WBPH increases earlier than BPH in the rice growing season. This could be a possible reason why insecticide resistance against fipronil occurred only on the WBPH species. The overuse of insecticides is often the precursor to the development of insecticide resistance and many Asian countries rely heavily upon a limited number of compounds for planthopper control (Nagata et al., 2002; Sun et al., 1996).

The present study suggests that the insecticide resistance of BPH against imidacloprid not occurred in the Philippines. This is because the BPH outbreaks has 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. In this reason, the insecticide susceptibility of WBPH against fipronil in the Philippines was as low as those in East-Asia and Vietnam.

In the Mekong Delta of southern Vietnam, the outbreaks of the two BPH-transmitted virus diseases, rice ragged stunt virus (RRSV) and rice glassy stunt virus (RGSV), have occurred since 2005, resulting in the heavily use of insecticides to control BPH. The present study showed that the $LD_{50}$-values in two southern Vietnam BPH populations tended to be larger than those in the other locations for BPMC, imidacloprid, and fipronil. Thus, it should be continued carefully to monitor the status of insecticide susceptibility in BPH against these insecticides in southern Vietnam and neighboring countries such as Thailand.

Based on the virulent spectrum, the current Asian BPH populations were classified into three groups: The first group: East Asian BPH populations from Taiwan, China, Japan and northern Vietnam were virulent to Mudgo (*Bph1*) and ASD7 (*bph2*), while avirulent to the other four varieties. The second group: Southeast Asian BPH populations from Philippines were virulent to Mudgo (*Bph1*), ASD7 (*bph2*), and also partially virulent to Babawee (*bph4*). The third group: BPH populations from southern Vietnam were virulent to Mudgo (*Bph1*), ASD7 (*bph2*) and also involving quite high percentage of the BPH females were virulent to Babawee (*bph4*).

The present results showed that the virulent status of the BPH populations from southern Vietnam is higher than that from northern Vietnam. In Vietnam, rice cropping system is quite different from north and south region. In Northern Vietnam, Red River Delta region, rice is cultivated two times per year. In southern Vietnam, especially in the Mekong River Delta region; high-yielding improved rice varieties are cultivated all year round. Thus, the BPH population became adapted to cultivated rice varieties in this area. This adaptation may have been accelerated under conditions of insecticide overuse.

Present study demonstrates that the varieties Rathu Heenati and Balamawee have a broad spectrum of resistance against all the Asian BPH populations tested. This result agrees well with the previous studies that Rathu Heenati and Balamawee carried multiple resistance genes against the BPH (Sun et al., 2005; Jairin et al., 2007). These multiple genes help maintain their durable resistance to BPH. To avoid the development of virulent biotypes, considerable effort is being devoted to developing strategies such as the sequential release of resistance genes and use of pyramided lines carrying multiple genes in an attempt to control the BPH.

## References

Endo, S., Tsurumachi, M. (2001) Insecticide susceptibility of the brown planthopper and the white-backed planthopper collected from southeast Asia. J. Pesticide Sci. 26: 82-86.

Fukuda, H., Nagata, T. (1969) Selective toxicity of several insecticides on three planthoppers. Jpn. J. Appl. Entomol. Zool. 13: 142-149.

Gallagher, K. D., Kenmore, P. E., Sogawa, K. (1994) Judicial use of insecticides deter planthopper outbreaks and extend the life of resistant varieties in Southeast Asian rice. *In*: Denno, R. F., Perfect, T. J. (eds.), Planthoppers: Their Ecology and management. Chapman and Hall, New York, 599-614.

Jairin, J., Phengrat, K., Teangdeerith, S., Vanavichit, A., Toojinda, T. (2007) Mapping of a broad-spectrum brown planthopper resistance gene, *Bph3*, on rice chromosome 6. Mol. Breeding 19: 35-44. DOI: 10.1007/s11032-006-9040-3

Kisimoto R. (1976) Synoptic weather conditions inducing long-distance immigration of planthoppers, *Sogatella furcifera* Horváth and *Nilaparvata lugens* Stål. Ecol. Entomol. 1: 95-109. DOI: 10.1111/j.1365-2311.1976.tb01210.x

Khush, G. S. (1979) Genetics of and breeding for the resistance to the brown planthopper. *In*: Brown Planthopper: Threat to Rice Production in Asia. International Rice Research Institute (IRRI), Los Baños, Philippines, 321-332.

Liu, Z., Han, Z., Wang, Y., Zhang, L., Zhang, H., Liu, C. (2003a) Selection for imidacloprid resistance in *Nilaparvata lugens*: cross-resistance patterns and possible mechanisms. Pest Manag. Sci. 59: 1355-1359. DOI: 10.1002/ps.768

Liu, Z., Wang, Y., Han, Z., Li, G., Deng, Y., Tian, X., Nagata, T. (2003b) Comparision of the susceptibilities of *Nilaparvata lugens* and *Sogatella furcifera* from three areas to ten insecticides. J. Nanjing Agric. Univ. 26: 29-32.

Matsumura, M., Takeuchi, H., Satoh, M., Sanada-Morimura, S., Otuka, A., Watanabe, T., Dinh, T. V. (2008) Species-specific insecticide resistance to imidacloprid and fipronil in the rice planthoppers, *Nilaparvata lugens* and *Sogatella furcifera*, in East and Southeast Asia. Pest Manag. Sci. 64: 1115-1121. DOI: 10.1002/ps.1641

Nagata, T., Kamimuro, T. (2002) Joint survey on insecticide susceptibility of the migratory rice planthoppers in Asian countries. Plant Prot. 56: 488-491.

Nagata, T., Kamimuro, T., Wang, Y. C., Han, S. G., Noor, N. M. (2002) Recent status of insecticide resistance of long-distance migrating rice planthoppers monitored in Japan, China, and Malaysia. J. Asia-Pacific Entomol. 5: 113-116.

Ping, X., Endo, S., Suzuki, K., Ohtsu, K. (2001) The insecticide susceptibility of the brown planthopper, *Nilaparvata lugens*, and white-backed planthopper, *Sogatella furcifera*, collected from China and Japan. Kyushu Plant Prot. Res. 47: 54-57.

Sogawa, K. (1982) The rice planthopper: Feeding physiology and host plant interactions. Annu. Rev. Entomol. 27: 49-73. DOI: 10.1146/annurev.en.27.010182.000405

Sogawa, K. (1992a) A change in biotype property of brown planthopper populations immigrating into Japan and their probable source areas. Proc. Assoc. Plant Prot. Kyushu 38: 63-68.

Sogawa, K. (1992b) Rice brown planthopper (BPH) immigrants in Japan change biotype. Int. Rice Res. Newsl. 17(2): 26-27.

Sun, J. Z., Fan, J. C., Xia, L. R. (1996) Studies on the insecticidal activity of imidacloprid and its application in paddy fields against the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae). Acta Entomol. Sinica 39: 37-45.

Sun, L., Su, C., Wang, C., Zhai, H., Wan, J. (2005) Mapping of a major resistance gene to the brown planthopper in the rice cultivar Rathu Heenati. Breed. Sci. 55: 391-396. DOI: 10.1270/jsbbs.55.391

Tanaka, K. (1999) Recent status in virulence to resistant rice varieties of brown planthopper

*Nilaparvata lugens* immigrating into Japan. Annu. Rept. Kanto-Tosan Plant Prot. Soc. 46: 85-88.
Tanaka, K. (2000) A simple method for evaluating the virulence of the brown planthopper. IRRN 25(1): 18-19.
Tanaka, K., Matsumura, M. (2000) Development of virulence to resistant varieties in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), immigrating into Japan. Appl. Entomol. Zool. 53(4): 529-533. DOI: 10.1303/aez.2000.529