

Current status of insecticide resistance in the small brown planthopper, *Laodelphax striatellus*, in Japan, Taiwan, and Vietnam

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Abstract The small brown planthopper, *Laodelphax striatellus*, is one of the most serious pest insects of rice plants. A large migration of the insects from overseas was reported in western parts of Japan in June 2008. Insecticide resistance to imidacloprid, fipronil and BPMC was compared among local populations in these western regions after migration. The insecticides were applied to the insects using a topical application method. In some populations, the resistance status coincided with that of the immigrant insects just after migration, i.e., resistance to imidacloprid but susceptibility to fipronil. In other populations, resistance was observed not only against imidacloprid but also fipronil. It is likely that the status of the latter populations resulted from intercrossing between domestic populations of the insects and migrants. Insecticide resistance was also assessed in other areas of northern and eastern parts of Japan. In general, these

populations showed relatively low resistance, although resistance to fipronil was high in the eastern part of Japan where the density of domestic populations has recently increased. Insecticide susceptibilities were also assessed in several sites in Taiwan and the northern parts of Vietnam. Although susceptibilities differed among these sites or countries, they have recently seen a decline for all three insecticides.

Keywords Imidacloprid · Fipronil · BPMC · Long-distance migration · Topical application

Introduction

The small brown planthopper, *Laodelphax striatellus* (Fallén), is one of the most important pest insects of rice and wheat crops in Asia. This species can overwinter in temperate and cold temperate zones, which include almost all of Japan, Taiwan and China. This species does not seriously damage the rice plant when feeding, unlike other planthoppers, such as the brown planthopper, *Nilaparvata lugens* (Stål) or the whitebacked planthopper, *Sogatella furcifera* (Horváth); however, *L. striatellus* can transfer the *Rice stripe virus* to host plants, which then develop rice stripe disease (Shinkai 1962).

The first description of serious damage to rice crops by rice stripe disease was reported in Japan in 1903 (Kuribayashi 1931). Subsequently, there were outbreaks of the disease during the 1980s (Shinkai 1985). From the late 1990s, the rice stripe disease problem disappeared in Japan following the development of an effective insecticide treatment regime for seedling boxes using imidacloprid or fipronil that controlled pest insects, including *L. striatellus*. Recently, however, the density of *L. striatellus* has gradually increased each year, and

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the frequency of viruliferous insects carrying the *Rice stripe virus* has also increased in the western part of Japan, mainly in the Kyushu region (Matsumura and Otuka 2009). As the density of *L. striatellus* has increased in these areas, so also has resistance to fipronil (Matsumura and Otuka 2009; Otuka et al. 2010). Outbreaks of *L. striatellus* have also occurred in the eastern part of China, including Jiangsu and Zhejiang Provinces, since the mid-2000s (Sogawa 2005; Wang et al. 2008; Wei et al. 2009). In these areas, considerable quantities of insecticides have been applied in attempts to control *L. striatellus*, and the development of insecticide resistance to imidacloprid has been reported (Ma et al. 2007).

It was long thought that *L. striatellus* did not undertake long-distance, overseas migration (Ohkubo 1981; Hoshizaki 1997). Although large-scale migration has not been observed in *L. striatellus*, a small number of *L. striatellus* has been captured together with a large number of *S. furcifera* on an ocean weather ship on the East China Sea every year (Matsumura and Otuka 2009) and a right trap in Ureshino, Saga prefecture (Kuchiki and Ogata, unpublished data) in the rainy season (June to July). This evidence suggests that *L. striatellus* has the potential to undertake long-distance migration. On 5 June 2008, large-scale overseas migration was detected in the western part of Japan, and the source population was estimated to be Jiangsu Province in China by backward trajectory analysis (Otuka et al. 2010). The immigrant populations were resistant to imidacloprid, as are the Jiangsu populations, and thus differed from the Japanese domestic populations that showed resistance only to fipronil (Otuka et al. 2010). Although *L. striatellus* with imidacloprid resistance should have migrated from China before 2008, the insects had probably not resulted in a large impact on domestic populations because the number of migrants was too small. On 5 June 2008, however, the presence of a huge number of migrant insects had the potential to cause serious problems in the western part of Japan if the different populations interbred and the resulting hybrid population showed resistance to both insecticides. In the present study, insecticide resistance of *L. striatellus* was monitored in the western part of Japan, mainly in the Kyushu region that received an overseas migration in June 2008. Insecticide resistance was also examined in the eastern and northern parts of Japan, Taiwan and the northern part of Vietnam. The effects of overseas migration of *L. striatellus* are discussed in terms of the development of insecticide resistance.

Materials and methods

Collection sites in the western part of Japan

Mass overseas migration to the western part of Japan, mainly the Kyushu region, was observed on 5 June 2008

(Otuka et al. 2010). We collected *L. striatellus* populations in the western part of Japan, including one site in Yamaguchi Prefecture (Japan-YM), two sites in Nagasaki Prefecture (Japan-NGN and Japan-NGS), one site in Fukuoka Prefecture (Japan-FKJ) and three sites in Kumamoto Prefecture (Japan-KMO, Japan-KME and Japan-KMT) from June to September 2008 (Fig. 1; Table 1). Data on one site in Kumamoto Prefecture (Japan-KMK) in 2008 were obtained from Otuka et al. (2010). To compare insecticide resistance in these populations after overwintering, the same sites were also sampled from March to May 2009. In addition, *L. striatellus* was collected at two new sites in Fukuoka Prefecture (Japan-FKY and Japan-FKO) and another site in Saga Prefecture (Japan-SGK) from April to June 2009.

Collection sites in the eastern and northern parts of Japan

Overseas immigration of *L. striatellus* in 2008 affected only the western parts of Japan, including the Kyushu region and Yamaguchi and Shimane Prefectures, but not the eastern or northern parts (Otuka et al. 2010). We compared insecticide resistance in populations unaffected by migration with those in western Japan. In 2009, we sampled populations from two sites in the Tochigi Prefecture (Japan-TGOT and Japan-TGOY) in the eastern part of Japan, and in September 2009, we sampled three sites in Hokkaido Prefecture (Japan-HKS, Japan-HKI and Japan-HKN) in the northern part of Japan (Fig. 1; Table 1).

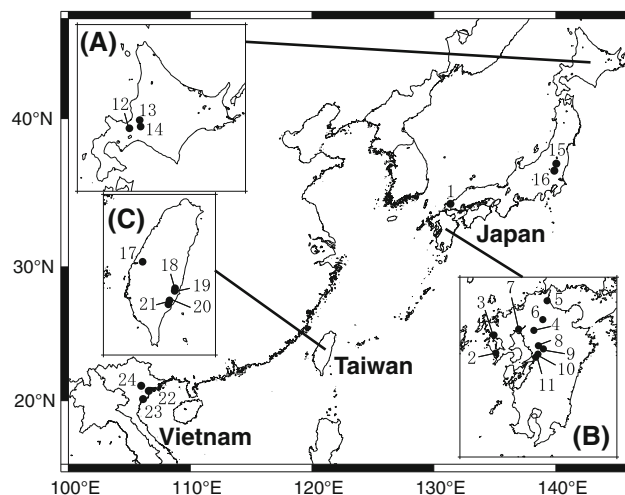


Fig. 1 Map locations of the populations investigated in this study. The sites are numbered, and the names of each site are given in Table 1. *Insets* show **a** Hokkaido, **b** Kyushu region in Japan and **c** Taiwan

Table 1 Locality and collection date of tested populations of *Laodelphax striatellus*

| No. ^a | Population | Locality | Lat/long | Collection date | | | |
|------------------|------------|--|-------------------|-----------------|--------|--------|--------|
| | | | | 2006 | 2007 | 2008 | 2009 |
| 1 | Japan-YM | Aburatani, Nagato, Yamaguchi, Japan | N 34.43, E 130.98 | | | Aug 20 | |
| 2 | Japan-NGN | Teguma, Nagasaki, Nagasaki, Japan | N 32.78, E 129.80 | | | Aug 29 | Mar 12 |
| 3 | Japan-NGS | Hario, Sasebo, Nagasaki, Japan | N 33.12, E 129.76 | | | Aug 29 | Mar 12 |
| 4 | Japan-FKJ | Joyo, Yame, Fukuoka, Japan | N 33.24, E 130.67 | | | Jun 17 | Apr 02 |
| 5 | Japan-FKY | Yukuhashi, Fukuoka, Japan | N 33.71, E 130.95 | | | | Apr 02 |
| 6 | Japan-FKO | Ochiai, Soeda, Fukuoka, Japan | N 33.49, E 130.87 | | | | Apr 02 |
| 7 | Japan-SGK | Kawazoe, Saga, Japan | N 33.23, E 130.31 | | | | Jun 05 |
| 8 | Japan-KMK | Aioi, Koshi, Kumamoto, Japan | N 32.92, E 130.75 | | | | May 21 |
| 9 | Japan-KMO | Shitamachi, Ozu, Kumamoto, Japan | N 32.86, E 130.86 | | | Sep 05 | May 21 |
| 10 | Japan-KME | Ezu, Kumamoto, Kumamoto, Japan | N 32.76, E 130.74 | | | Sep 05 | Mar 23 |
| 11 | Japan-KMT | Tomiai, Kumamoto, Kumamoto, Japan | N 32.70, E 130.69 | | | Sep 05 | Mar 23 |
| 12 | Japan-HKS | Hitsujigaoka, Sapporo, Hokkaido, Japan | N 43.01, E 141.41 | | | | Sep 01 |
| 13 | Japan-HKI | Iwamizawa, Hokkaido, Japan | N 43.24, E 141.72 | | | | Sep 02 |
| 14 | Japan-HKN | Naganuma, Hokkaido, Japan | N 43.05, E 141.76 | | | | Sep 02 |
| 15 | Japan-TGOT | Tonouchi, Ohtawara, Tochigi, Japan | N 36.89, E 140.03 | | | | Sep 24 |
| 16 | Japan-TGOY | Matsunuma, Oyama, Tochigi, Japan | N 36.35, E 139.78 | | | | Sep 28 |
| 17 | Taiwan-YL | Shiluo, Yunlin, Taiwan | N 23.86, E 120.48 | May 30 | | | |
| 18 | Taiwan-HLA | Fuli, Hualien, Taiwan | N 23.19, E 121.28 | Oct 18 | May 31 | | |
| 19 | Taiwan-HLB | Fuli, Hualien, Taiwan | N 23.25, E 121.29 | | | | Oct 14 |
| 20 | Taiwan-TTG | Guanshan, Taitung, Taiwan | N 23.02, E 121.18 | Oct 18 | May 30 | | Oct 14 |
| 21 | Taiwan-TTL | Luye, Taitung, Taiwan | N 22.95, E 121.16 | | | | Oct 14 |
| 22 | Vietnam-HP | An Lao, Hai Phong, Vietnam | N 20.77, E 106.60 | | May 04 | | |
| 23 | Vietnam-HD | Thai Duong, Binh Giang, Hai Duong, Vietnam | N 20.14, E 106.12 | | | | Sep 10 |
| 24 | Vietnam-BN | Tam Son, Tu Son, Bac Ninh, Vietnam | N 21.15, E 105.98 | | | | Sep 11 |

^a See Fig. 1 for the location of the collection sites

Collection sites in Taiwan and northern part of Vietnam

Laodelphax striatellus populations were sampled from one site in the western part (Taiwan-YL) and four sites of the eastern part (Taiwan-HLA, Taiwan-HLB, Taiwan-TTG and Taiwan-TTL) of Taiwan from 2006 to 2009 (Fig. 1; Table 1). Populations at three sites in the northern part of Vietnam (Vietnam-HP, Vietnam-HD and Vietnam-BN) were also sampled in 2007 and 2009.

Insecticide susceptibility

Each population sample consisted of more than 100 adults with the same number of males and females. The sampled populations were maintained in the laboratory for 2–5 generations prior to testing and were kept on rice seedlings (var. Reihou) at 25°C under 16L8D. Insecticide susceptibility was determined by a standard topical application method (Fukuda and Nagata 1969) using imidacloprid (98.5%), fipronil (90.7%) or BPMC (96.9%). Insecticide resistance for BPMC was examined in populations

collected in 2008, but not in those in 2009 from the western part of Japan. Imidacloprid was provided by Bayer Crop Science K.K. (Tokyo, Japan), fipronil by BASF Japan Ltd. (Tokyo, Japan) and BPMC by Sumitomo Chemical Co. Ltd. (Tokyo, Japan).

Long-winged adult females within 7 days after emergence were anesthetized with carbon dioxide for about 5 s prior to treatment. A 0.08- μ l droplet of acetone solution was applied topically to the dorsal surface of the thorax with a hand micro-applicator (Burkard Manufacturing Company Ltd., Hertfordshire, UK). The treated insects were kept in a transparent plastic box (5 cm diameter, 10 cm high) with rice seedlings as described above. Mortality was determined 24 h after treatment for all insecticides and also at 48 h for fipronil. A minimum of 45 females were tested at each insecticide concentration, and 5 or 6 different concentrations were tested. Average body weight was calculated for about 15 individuals in each population at each insecticide test and was used to convert the unit of LD₅₀ from μ g/insect to μ g/g. The average body weight of the overall tested populations was

1.13 ± 0.15 (SD) mg. The LD₅₀ values (µg/g), 95% confidence intervals and the slopes of the regression lines were calculated by Bliss's (1935) probit method with PoloPlus software (LeOra Software 2003).

Results

Western part of Japan

In populations from areas affected by overseas migration, the LD₅₀ values of imidacloprid ranged from 6.3 to 23.3 µg/g, and those of fipronil ranged from 0.17 to 0.44 µg/g at one site in Yamaguchi Prefecture (Japan-YM) and at two sites in Nagasaki Prefecture (Japan-NGN and Japan-NGS) in 2008 (Table 2). On the other hand, the LD₅₀ values of imidacloprid and fipronil ranged from 2.1 to 28.3 and 39.8 to 193.8 µg/g, respectively, at one site in Fukuoka Prefecture (Japan-FKJ) and three sites in Kumamoto Prefecture (Japan-KMK, Japan-KMO and Japan-KME) in 2008 (Table 2). Although a small LD₅₀ value for fipronil (0.31 µg/g) was found at one site in Kumamoto Prefecture (Japan-KMT), the slope of the regression was very low, indicating that resistance to fipronil has been increasing in recent years. The LD₅₀ for BPMC ranged from 114.5 to 474.0 µg/g at all sites in the western part of Japan (Table 2).

In the populations sampled after overwintering, the LD₅₀ values were similar to those of the previous year at all sites in the migration areas, i.e., at two sites in Nagasaki Prefecture (Japan-NGN and Japan-NGS), one site in

Fukuoka Prefecture (Japan-FKJ) and four sites in Kumamoto Prefecture (Japan-KMK, Japan-KMO, Japan-KME and Japan-KMT). At three new sites sampled in 2009, two sites in Fukuoka Prefecture (Japan-FKY and Japan-FKO) and one site in Saga Prefecture (Japan-SGK), the LD₅₀ values for imidacloprid and fipronil ranged from 1.9 to 6.2 and 7.5 to 24.8 µg/g, respectively (Table 3).

Eastern and northern parts of Japan

The LD₅₀ values for fipronil ranged from 49.3 to 493.8 µg/g at two sites in Tochigi Prefecture (Japan-TGOT and Japan-TGOY) in 2009 (Table 4). In contrast, the LD₅₀ values for fipronil ranged from 0.05 to 0.06 µg/g at three sites in Hokkaido Prefecture (Japan-HKS, Japan-HKI and Japan-HKN). The LD₅₀ values for imidacloprid ranged from 0.18 to 0.57 µg/g at all sites in both Hokkaido and Tochigi Prefectures (Table 4). The LD₅₀ value for BPMC ranged from 13.7 to 29.9 µg/g at three sites in Hokkaido and two sites in Tochigi Prefectures (Table 4).

Taiwan and northern part of Vietnam

The LD₅₀ values ranged from 0.40 and 1.4 µg/g for imidacloprid, and 0.06 and 0.28 µg/g for fipronil in the western and eastern parts of Taiwan in 2006 and 2007 (Table 5). However, the LD₅₀ values for imidacloprid and fipronil were increased in 2009 (Table 5). In the northern part of Vietnam, the LD₅₀ values for imidacloprid and fipronil were 3.0 and 0.11 µg/g, respectively, in 2007, but had increased by 2009 (Table 5). The LD₅₀ value for

Table 2 LD₅₀ values (µg/g) of *Laodelphax striatellus* populations collected in 2008 in the western part of Japan

| Population ^a | Imidacloprid | | | Fipronil | | | | BPMC | | | |
|--------------------------|------------------|----------|-----------------------|------------------|----------|------------------|----------|-----------------------|------------------|----------|-----------------------|
| | LD ₅₀ | <i>b</i> | <i>G</i> ^b | 24 h | | 48 h | | <i>G</i> ^b | LD ₅₀ | <i>b</i> | <i>G</i> ^b |
| | | | | LD ₅₀ | <i>b</i> | LD ₅₀ | <i>b</i> | | | | |
| Japan-YM | 7.1 (1.6–15.3) | 1.3 | 3 | 0.17 (0.15–0.20) | 4.1 | 0.08 (0.06–0.10) | 2.5 | 3 | 169.4 (133–223) | 2.2 | 3 |
| Japan-NGN* | 23.3 (16.6–33.3) | 1.4 | 2 | 0.44 (0.34–0.61) | 2.0 | 0.42 (0.30–0.76) | 1.3 | 2 | 321.1 (277–377) | 3.5 | 2 |
| Japan-NGS* | 6.3 (4.3–9.1) | 1.2 | 2 | 0.35 (0.27–0.49) | 1.7 | 0.19 (0.12–0.30) | 1.0 | 2 | 180.0 (151–216) | 2.5 | 2 |
| Japan-FKJ* | 15.8 (10.9–22.6) | 1.3 | 2 | 81.9 (23.7–2254) | 0.4 | 0.11 (0.08–0.15) | 1.5 | 2 | 177.9 (151–212) | 3.4 | 2 |
| Japan-KMK ^c * | 2.1 (1.3–4.6) | 1.3 | 2 | 39.8 (15.0–184) | 0.4 | 2.7 (1.1–5.5) | 0.5 | 2 | 114.5 (91.3–142) | 2.6 | 2 |
| Japan-KMO* | 4.0 (2.7–5.7) | 1.2 | 2 | 193.8 (81.4–802) | 0.5 | 8.4 (4.1–15.9) | 0.6 | 2 | 457.9 (404–522) | 5.0 | 2 |
| Japan-KME* | 28.3 (20.4–41.0) | 1.6 | 2 | 187.9 (88.5–589) | 0.6 | 8.9 (4.5–16.6) | 0.6 | 2 | 474.0 (415–547) | 4.6 | 2 |
| Japan-KMT* | 8.7 (5.3–13.6) | 1.1 | 2 | 0.31 (0.07–0.76) | 0.5 | 0.59 (0.33–0.99) | 0.6 | 2 | 311.3 (265–375) | 3.4 | 2 |

LD₅₀ value and its 95% confidence interval in parentheses are shown in µg/g, and the slope of the regression line (*b*) is shown

All LD₅₀ values were determined 24 h after treatment, except for Fipronil (24 and 48 h)

^a Asterisk shows that the population was collected at the same location in 2009. See Table 3

^b Generations prior to topical tests

^c Data from Otuka et al. (2010)

Table 3 LD₅₀ values (μg/g) of *Laodelphax striatellus* populations collected in 2009 in the western part of Japan

| Population ^a | Imidacloprid | | | Fipronil | | | | |
|-------------------------|------------------|----------|----------------|-------------------|----------|------------------|----------|----------------|
| | LD ₅₀ | <i>b</i> | G ^b | 24 h | | 48 h | | G ^b |
| | | | | LD ₅₀ | <i>b</i> | LD ₅₀ | <i>b</i> | |
| Japan-NGN* | 16.4 (11.4–25.1) | 1.3 | 3 | 0.46 (0.34–0.70) | 1.8 | 0.32 (0.24–0.46) | 1.6 | 3 |
| Japan-NGS* | 13.9 (10.4–18.4) | 2.0 | 3 | 0.22 (0.18–0.27) | 2.3 | 0.11 (0.09–0.14) | 2.1 | 3 |
| Japan-FKJ* | 13.6 (9.9–19.1) | 1.7 | 2 | 282.6 (89.2–4418) | 0.4 | 2.1 (0.93–3.9) | 0.4 | 2 |
| Japan-FKY | 6.2 (3.9–10.1) | 1.1 | 2 | 24.8 (14.1–56.8) | 1.0 | 10.4 (5.7–23.2) | 0.8 | 2 |
| Japan-FKO | 1.9 (1.2–2.8) | 1.3 | 2 | – ^c | | – ^c | | |
| Japan-SGK | 4.1 (2.7–7.8) | 1.1 | 3 | 7.5 (3.0–14.3) | 0.7 | 0.3 (0.07–0.8) | 0.6 | 3 |
| Japan-KMK* | 4.0 (2.4–7.3) | 0.9 | 1 | 12.4 (7.3–22.9) | 0.9 | 3.4 (2.2–5.0) | 0.5 | 1 |
| Japan-KMO* | 3.9 (1.8–7.6) | 0.7 | 4 | 956.3 (121–) | 0.3 | 16.2 (7.7–37.5) | 0.5 | 1 |
| Japan-KME* | 9.2 (6.3–17.8) | 1.3 | 1 | 18.3 (10.2–40.6) | 0.8 | 2.7 (1.8–4.1) | 1.1 | 1 |
| Japan-KMT* | 6.5 (4.6–9.1) | 1.5 | 1 | 14.1 (7.3–32.3) | 0.6 | 3.2 (1.4–6.1) | 0.7 | 1 |

LD₅₀ value and its 95% confidence interval in parentheses are shown in μg/g, and the slope of the regression line (*b*) is shown

All LD₅₀ values were determined 24 h after treatment, except for Fipronil (24 and 48 h)

^a Asterisk shows that the population was collected at the same location in 2008. See Table 2

^b Generations prior to topical tests

^c Not observed

Table 4 LD₅₀ values (μg/g) of *Laodelphax striatellus* populations collected in 2009 in the eastern and northern parts of Japan

| Population | Imidacloprid | | | Fipronil | | | | BPMC | | | |
|------------|------------------|----------|----------------|------------------|----------|------------------|----------|------------------|------------------|----------|----------------|
| | LD ₅₀ | <i>b</i> | G ^a | 24 h | | 48 h | | G ^a | LD ₅₀ | <i>b</i> | G ^a |
| | | | | LD ₅₀ | <i>b</i> | LD ₅₀ | <i>b</i> | | | | |
| Japan-HKS | 0.18 (0.16–0.22) | 4.4 | 3 | 0.05 (0.04–0.06) | 2.5 | – ^b | 2 | 24.9 (18.8–33.7) | 1.7 | 2 | |
| Japan-HKI | 0.29 (0.2–0.3) | 3.1 | 2 | 0.06 (0.05–0.07) | 3.2 | – ^b | 2 | 13.7 (9.7–17.8) | 1.8 | 2 | |
| Japan-HKN | 0.21 (0.2–0.3) | 3.4 | 3 | 0.05 (0.04–0.06) | 2.2 | – ^b | 2 | 26.6 (21.3–33.0) | 2.6 | 2 | |
| Japan-TGOT | 0.27 (0.21–0.35) | 1.7 | 2 | 49.3 (33.5–72.5) | 1.4 | 5.4 (1.9–9.0) | 1.4 | 20.1 (17.5–25.9) | 2.4 | 1 | |
| Japan-TGOY | 0.57 (0.46–0.71) | 2.2 | 1 | 493.8 (196–2265) | 0.4 | 1.1 (0.2–3.2) | 0.5 | 29.9 (24.7–36.9) | 2.2 | 2 | |

LD₅₀ value and its 95% confidence interval in parentheses are shown in μg/g, and the slope of the regression line (*b*) is shown

All the LD₅₀ values were determined 24 h after treatment, except for Fipronil (24 and 48 h)

^a Generations prior to topical tests

^b Not observed

BPMC ranged from 128.7 to 262.2 μg/g at all sites in Taiwan and in the northern part of Vietnam in 2008 and 2009.

At 48 h of treatment, although the LD₅₀ values for fipronil became lower at all sites compared to those obtained after 24 h, their regression slopes maintained similar values at both 24 and 48 h (Tables 2, 3, 4, 5).

Discussion

In the previous study, the LD₅₀ values for imidacloprid were reported to range from 0.14 to 0.83 μg/g in several populations collected in Kyushu region during 1991–1992 (Endo and Tsurumachi 2000; Sone et al. 1995). These

values were regarded as the baseline susceptibility for imidacloprid, i.e., the value of a susceptible population, in *L. striatellus* in the western part of Japan, because this insecticide began to be used in the early 1990s. The LD₅₀ value of BPMC was reported to be 7.8 μg/g for a local population collected in Kumamoto Prefecture in 1978 (Takita 1979). Another previous study also showed that the LD₅₀ value of BPMC was 6.6 μg/g in a local population in Kagawa Prefecture in 1969 (Sasaki and Ozaki 1976), which is not in Kyushu but in a neighboring region. These values were also regarded as the baseline susceptibility for BPMC in *L. striatellus* in the western part of Japan because BPMC has been used since the early 1970s. Although there are no previous data on the LD₅₀ value for fipronil in *L. striatellus*, the baseline susceptibility for fipronil could

Table 5 LD₅₀ values (μg/g) of *Laodelphax striatellus* populations collected in 2006–2009 in Asia

| Population | Collection Year | Imidacloprid | | | Fipronil | | | | BPMC | | | |
|------------|-----------------|------------------|----------|----------------|--------------------|----------|------------------|----------|----------------|------------------|----------|----------------|
| | | LD ₅₀ | <i>b</i> | G ^a | 24 h | | 48 h | | G ^a | LD ₅₀ | <i>b</i> | G ^a |
| | | | | | LD ₅₀ | <i>b</i> | LD ₅₀ | <i>b</i> | | | | |
| Taiwan-YL | 2006 | 0.40 (0.05–0.87) | 0.5 | 3 | <0.28 ^b | | – ^c | | 3 | 176.6 (85.3–442) | 2.4 | 3 |
| Taiwan-HLA | 2006 | 1.4 (1.08–1.95) | 1.5 | 4 | 0.06 (0.04–0.09) | 1.8 | – ^c | | 3 | 227.9 (186–288) | 3.1 | 3 |
| Taiwan-HLA | 2007 | 0.40 (0.31–0.52) | 1.8 | 3 | 0.24 (0.18–0.31) | 1.7 | – ^c | | 3 | 128.9 (101–159) | 2.5 | 3 |
| Taiwan-HLB | 2009 | 10.9 (6.8–18.6) | 0.9 | 2 | 4.7 (2.8–9.1) | 0.9 | 1.7 (1.0–2.8) | 0.9 | 3 | 507.6 (428–603) | 3.2 | 3 |
| Taiwan-TTG | 2006 | 1.9 (1.41–2.75) | 1.3 | 4 | 0.11 (0.04–0.23) | 1.2 | – ^c | | 3 | 441.7 (323–563) | 2.8 | 3 |
| Taiwan-TTG | 2007 | 2.2 (1.85–2.73) | 2.5 | 3 | 0.32 (0.22–0.46) | 1.6 | – ^c | | 3 | 307.1 (173–777) | 2.2 | 3 |
| Taiwan-TTG | 2009 | 4.8 (3.0–705) | 0.9 | 2 | 3.2 (2.1–5.8) | 1.1 | 7.6 (1.1–) | 0.5 | 2 | 305.6 (247–377) | 2.4 | 3 |
| Taiwan-TTL | 2009 | 8.2 (5.3–13.2) | 1.0 | 2 | 1.1 (0.8–1.7) | 1.1 | 0.5 (0.3–4.8) | 0.7 | 2 | 293.7 (247–349) | 3.3 | 3 |
| Vietnam-HP | 2007 | 3.0 (1.94–4.44) | 1.5 | 3 | 0.11 (0.09–0.12) | 1.7 | – ^c | | 3 | 128.7 (102–163) | 2.3 | 3 |
| Vietnam-HD | 2009 | 10.2 (7.5–13.5) | 1.8 | 3 | 98.3 (48.5–314) | 0.7 | 2.2 (0.97–3.9) | 0.8 | 3 | 262.2 (218–319) | 2.6 | 3 |
| Vietnam-BN | 2009 | 2.2 (1.4–6.0) | 1.1 | 3 | 171.2 (75.7–192) | 0.6 | 2.6 (0.71–5.5) | 0.7 | 3 | 163.5 (136–192) | 3.6 | 4 |

LD₅₀ value and its 95% confidence interval in parentheses are shown with μg/g, and the slope of the regression line (*b*) is shown

All LD₅₀ values were determined 24 h after treatment, except for Fipronil (24 and 48 h)

^a Generations prior to topical tests

^b LD₅₀ value was not determined because mortality was higher than 50% in all treatments. The value is shown as the minimum dose treated

^c Not observed

be around 0.05 μg/g in three local populations in Hokkaido Prefecture, because these were the lowest values in our data.

A large migration of *L. striatellus* from overseas was observed in the western part of Japan, mainly to the Kyushu region and Yamaguchi Prefecture, in early June 2008 (Otuka et al. 2010). Immigrant populations sampled just after arrival in Kyushu were resistant to imidacloprid, but not fipronil; these resistance characteristics are identical to those of their source populations in Jiangsu Province, China (Otuka et al. 2010). Prior to this migration event in 2008, many researchers at prefectural-agricultural research institutes had reported the low LD₅₀ value of imidacloprid in the Kyushu region. For example, LD₅₀ values of imidacloprid ranged from 0.88 to 1.88 in Kumamoto Prefecture in 2006 (K. Nishimoto, unpublished data). The LD₅₀ values of imidacloprid were lower than 1.0 μg/g at 5 sites in each of Fukuoka (E. Murakami, unpublished data) and Saga Prefectures (F. Kuchiki, unpublished data) in 2006. These values were not so different from the baseline data described above (Endo and Tsurumachi 2000; Sone et al. 1995). These results suggest that LD₅₀ values of imidacloprid have not increased for a long period in the Kyushu region. On the other hand, prefectural agricultural research institutes reported that LD₅₀ values of fipronil were increased more than 100 times at several sites in Saga (F. Kuchiki, unpublished data), Kumamoto (Nishimoto, unpublished data) and Fukuoka (E. Murakami, unpublished

data) Prefectures in 2006, higher than the baseline data described above. The present study, however, shows that the LD₅₀ values are now high for imidacloprid, but not fipronil, in Nagasaki and Yamaguchi Prefectures (Table 2). One possible reason might be that these traits, i.e., resistance to imidacloprid and susceptibility to fipronil, directly reflected those of the migratory populations for at least 3 months following migration from overseas. The densities of domestic populations had been consistently low in Nagasaki Prefecture before the arrival of overseas migrants (R. Ohtsu, unpublished data). It is therefore possible that the immigrant populations could have freely colonized areas where few native insects were living. By contrast, LD₅₀ values for both imidacloprid and fipronil were high in Fukuoka and Kumamoto Prefectures (Table 2). In these areas, the densities of domestic populations had been relatively high since the early 2000s (T. Nakamura, unpublished data). The presence of a relatively dense local population may have reduced colonization only by the migrating insects. It is possible that insecticide resistance to imidacloprid or fipronil might spread as a result of intercrossing between immigrant and domestic populations in these areas. In laboratory populations, we found that intercrossing readily occurs between insects from immigrant and domestic populations (Sanada-Morimura et al., unpublished data). However, it will be necessary to elucidate the pattern of inheritance of insecticide resistance to imidacloprid and fipronil in order to accurately predict the

potential consequences of intercrossing between immigrant and domestic populations with regard to the spread of insecticide resistance.

During recent years, Chinese farmers have usually used imidacloprid but not fipronil for outbreaks of *L. striatellus* and *Rice stripe virus* because the former is cheaper than the latter (Sogawa 2005). Overuse of this insecticide might result in strong resistance to imidacloprid in China. On the other hand, both imidacloprid and fipronil are commonly used as nursery box insecticidal treatment to control rice planthoppers in rice paddy fields in the western part of Japan. It is unclear why the insecticide resistance of *L. striatellus* developed in Japan. It is necessary to determine why *L. striatellus* developed resistance only to fipronil in Japan.

The LD₅₀ values of BPMC ranged from 114.5 to 474.0 µg/g in the Kyushu region (Table 2). These values were 15–50 times larger than the baseline data described above. Similar large LD₅₀ values were reported for the Kyushu region in the early 1980s and early 1990s (230.1–540.1 µg/g in 6 sites in Nagasaki Prefecture, Ogawa 1987; 320 and 410 µg/g in two sites in Kumamoto Prefecture, Endo and Tsurumachi 2000), suggesting that insecticide resistance in this population to BPMC has not changed over several decades.

Although domestic populations of *L. striatellus* are able to overwinter in Japan by diapausing as third- or fourth-instar nymphs (Ito and Okada 1985; Noda 1990), the population densities usually decrease markedly during the winter season. If immigrant insects are more sensitive to environmental conditions such as cold temperatures and host plant species in winter than native populations, later generations of migrant insects (or of their intercrossed descendants) may have higher mortality in winter. If this is the case, insecticide resistance in the overwintering populations might show a reduced influence from migrant insects the next spring. The present study, however, showed that the overwintering populations maintained the resistance traits present in the previous year in Nagasaki (Japan-NGN and Japan NGS), Fukuoka (Japan-FKJ) and Kumamoto Prefectures (Japan-KMK, Japan-KMO, Japan-KME and Japan-KMT) (Tables 2, 3). Otuka et al. (2010) suggested that migrating insects landed in the western part of Japan, including a very large region from Yamaguchi to Kagoshima Prefectures. Our results suggested that insecticide susceptibility had been strongly affected by immigrant populations over a large area, supporting the findings of Otuka et al. (2010).

While insecticide susceptibilities to imidacloprid, fipronil and BPMC have decreased in many local populations in the western part of Japan, those in populations from the eastern and northern parts of Japan remain unaffected. At three sites in Hokkaido Prefecture in the northern part of

Japan, the LD₅₀ values for fipronil were low in 2009 (Table 4) compared with the western part of Japan. Furthermore, the LD₅₀ values for BPMC and imidacloprid were low in Hokkaido Prefecture compared to other areas of Japan (Table 4) and were the same as the baseline data described above. Consequently, the Hokkaido populations were not so strongly resistant to all three insecticides tested. This effect was likely due to the fact that the population densities of three rice planthoppers (*N. lugens*, *S. furcifera* and *L. striatellus*) are not high in this region, and consequently insecticides are used infrequently in Hokkaido. Ogawa (1987) also reported that the LD₅₀ value for BPMC was very low at one site in Hokkaido Prefecture in 1985. Overall, the data indicate that the level of insecticide resistance to the three insecticides has not changed in Hokkaido for a long time.

The LD₅₀ values in Tochigi populations (Japan-TGOT and Japan-TGOY) were also much lower for imidacloprid and BPMC than in western parts of Japan, and were the same as the baseline data described above. The Tochigi populations, however, were resistant to fipronil (Table 4), as were those in the western part of Japan. Around the site in Tochigi Prefecture (Japan-TGOY), the rate of viruliferous insects carrying *Rice stripe virus* has increased gradually each year. This is principally due to the large-area cultivation of barley and wheat crops, which are available for feeding the first generation of *L. striatellus* after overwintering (K. Ikezawa, personal communication). Fipronil has been mainly applied to control the rice stem borer, *Chilo suppressalis* (Walker), and the rice skipper, *Parnara guttata guttata* (Bremer et Grey), in paddy fields in some areas of Tochigi Prefecture. The LD₅₀ values, however, were tested at only two sites of Tochigi Prefecture in the present study. Further tests will be needed to determine whether *L. striatellus* is strongly resistant to fipronil in the eastern parts of Japan.

High LD₅₀ values for BPMC but low values for imidacloprid and fipronil were found at all sites in Taiwan in 2006 and 2007 (Table 5); however, between 2006 and 2009, the LD₅₀ values increased slightly for imidacloprid and fipronil at two sites in the eastern part of Taiwan (Table 5). Unlike in Japan, farmers in Taiwan do not use nursery box insecticidal treatment to protect and control insect pests for early season rice. *Laodelphax striatellus* is not currently a serious pest insect that requires control in Taiwan; however, they might be affected by spraying of insecticides to control *N. lugens* and the rice leafhopper (*Cnaphalocrocis medinalis* Guenée) in paddy fields.

In the early 1990s, the LD₅₀ values for imidacloprid and BPMC were very low in northern Vietnam (Endo et al. 2002) and were the same as the baseline data. Our results, however, indicate that insecticide susceptibility to these compounds has decreased greatly in northern Vietnam (Table 5),

although our collection sites were not identical to those in the previous report. Furthermore, susceptibility to fipronil decreased gradually from 2007 to 2009. Since an outbreak of *L. striatellus* occurred in the north in 2009 (D. V. Thanh, unpublished data), it will clearly be necessary to closely monitor the status of insecticide resistance of *L. striatellus* in the future.

The present study indicates that susceptibility to insecticides varies among local populations within and among countries. As a typical example, the LD₅₀ value for fipronil was high, but not that of imidacloprid, in western parts of Japan before the migration of insects from overseas. Susceptibility to imidacloprid but not fipronil was low in Jiangsu, China (Otuka et al. 2010). This status of resistance of *L. striatellus* is clearly different from *N. lugens* and *S. furcifera*, which developed species-specific insecticide resistance to imidacloprid and fipronil in East and Southeast Asia (Matsumura et al. 2008). Overseas migration, however, could occur in *L. striatellus* when a large outbreak of insects, the timing of the harvest and strong monsoon winds occur simultaneously. Just such a combination of circumstances occurred on 5 June 2008 (Otuka et al. 2010). The present study suggests that overseas migration might markedly affect the status of insecticide resistance in populations over long distances, such as in different countries, and that the effects of migration could be maintained for long periods, at least until the following year. It is likely that the result of intercrossing between immigrant and domestic populations changes their insecticide resistance. However, the genetic basis of resistance in *L. striatellus* to the insecticides used here is unknown, although some genes that confer resistance to imidacloprid have been reported in *N. lugens* (Liu et al. 2005; Liu and Han 2006; Wang et al. 2009). It will be necessary to investigate the genetic systems of insecticide resistance to imidacloprid and fipronil, and to determine their exact status to aid the control of *L. striatellus* in East and Southeast Asia.

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