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Biotype Comparisons of the Brown Planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) Collected in Japan and the Indochina Peninsula

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We compared biotypes of the brown planthopper (BPH) populations collected in Japan and Indochina Peninsula in 1992 to investigate possible source areas of the planthoppers that invade Japan. We quantified the honeydew excreted by the insects on six standard rice varieties to determine their biotypes. Eight populations used were roughly classified into two groups according to virulence to ASD7 rice with a *bph2* resistant gene. Low proportions (10–30%) of planthoppers in Japanese and Red River Delta populations were capable of feeding on ASD7, unlike most planthoppers (80–93%) from the tropical Indochina Peninsula. On IR26, which has a *Bph1* resistant gene, Japanese and Red River Delta planthoppers excreted ca. 30–50% of the quantity of honeydew excreted by BPH feeding on TN1 (no resistant gene), indicating the moderate susceptibility of the former to these planthoppers. Tropical populations collected from Mekong Delta and Thailand had slightly higher virulence to IR26. Rath Heenati and Babawee with *Bph3* and *bph4* resistant genes, respectively, remained highly resistant to all populations except those of the Mekong Delta. As a result, the biotype of Japanese populations bore a close resemblance to that of Red River Delta populations.

Key words: *Nilaparvata lugens*, biotype, migration, honeydew, Indochina, Japan, Vietnam

INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* (STÅL), does not hibernate in Japan. It invades Japan every year in June and July from South China through monsoons (SOGAWA and WATANABE, 1992). Chinese entomologists showed that there is a large number of immigrants between late March and May entering southern China from the south (CHENG et al., 1979; National Cooperated Network of Forecast for Brown Planthopper, 1980). Therefore, the BPH population of South China probably originated with these immigrants, and the origin of Japanese BPH may be traced to some areas in the Indochina Peninsula.

The term "biotype" in BPH was often used as a general concept that applied both to individuals and to populations that shared degrees of virulence on different rice varieties (CLARIDGE and HOLLANDER, 1983). Various biotypes and their move-

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ments have been reported in Asian countries (IRRI, 1982; SAXENA and BARRION, 1985). Since various biotypes are distributed in time and space in Asia, biotype can be a natural marker of the local BPH population. SOGAWA (1992 a) investigated biotype of immigrants invading Kyushu, Japan to determine their origin, along with biotype information from Southeast and East Asia. This led to his conclusion that South China and North Vietnam were possible source areas for the immigrants into Japan.

Possession of the same biotype among BPH sub-populations at various sites, however, does not indicate equal population virulence. Biotype populations show great individual variation within the population and little genetic homogeneity (CLARIDGE and HOLLANDER, 1983). In addition, the methods used in determining biotypes vary among reports. Therefore, we collected BPH in 1992 in Kyushu and the Indochina Peninsula and compared the biotype composition of the populations to determine spatial distribution of the biotypes of the population invading Japan.

MATERIALS AND METHODS

Insects used. Eight BPH populations were collected in 1992 in the rice fields of the Indochina Peninsula and Kyushu, Japan: four tropical populations in the Muda area of Malaysia, the Central Plain of Thailand and the Mekong Delta of Vietnam (two populations); two subtropical populations in the Red River Delta of Vietnam; and two temperate populations in Kyushu, southern Japan. The insects collected were kept in plastic containers (ca. $30 \times 25 \times 28$ cm) and reared on BPH-susceptible rice seedlings (variety: Reiho or Kinuhikari) until the biotype tests. Collection sites and details of the tested insects are shown in Fig. 1 and Table 1.

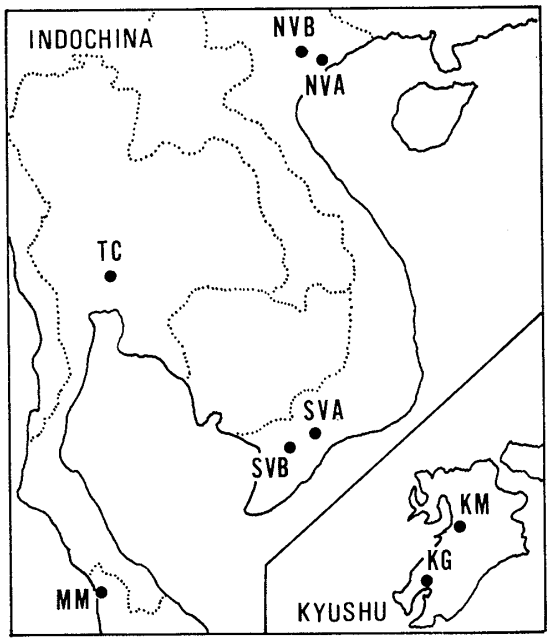

Fig. 1. Collection sites of the brown planthoppers used for the biotype experiment. KG: Kyushu A, KM: Kyushu B, NVA: Red River Delta A, NVB: Red River Delta B, SVA: Mekong Delta A, SVB: Mekong Delta B, TC: Thailand, MM: Malaysia.

Table 1. Brown planthopper populations used for the biotype experiment

Population	Symbol[a]	Collection		Generation used after collection
		Site	Time	
Kyushu A	KG	Kagoshima, Japan	1992 Jul.	next
Kyushu B	KM	Kumamoto, Japan	1992 Jul.	next
Red River Delta A	NVA	Hai Fun Prov., Vietnam	1992 Apr.	4–5
Red River Delta B	NVB	Hanoi, Vietnam	1992 Mar.	4–5
Mekong Delta A	SVA	Tien Giang Prov., Vietnam	1992 Apr.	4–5
Mekong Delta B	SVB	Hau Giang Prov., Vietnam	1992 Mar.	4–5
Thailand	TC	Suphan Buri Prov., Thailand	1992 Apr.	4–5
Malaysia	MM	Muda area, Malaysia	1992 Jan.	ca. 10

[a] See, Fig. 1.

Biotype identification method. Since honeydew excretion on rice reflects the feeding activity of BPH (PAGUIA et al., 1980; PARK and SONG, 1988), the quantity of honeydew can be used as an index of insect virulence. Honeydew excretion of individual insects feeding on standard rice varieties were measured.

We used the parafilm sachet method (HEINRICHS et al., 1985; SOGAWA, 1992 a) to quantify individual honeydew excretion. A parafilm sachet (ca. 30×25 mm) was set on a sheath of a rice plant. A newly emerged (within 24 h) female adult was confined in the sachet and was kept at 25°C for 48 h. After removing the insect, the weight of the honeydew was measured. The insects used were macropters. The Malaysian population was an exception in which about half of the test animals were newly emerged brachypterous female adults. The experiments were done twice (two replicates) for each population. For each variety ca. 25 insects (a total of 150 insects) were tested in an experiment.

We analyzed the data after subtracting 3 mg from each of the original quantities; original quantities of less than 3 mg were regarded as zero. When a sachet was set on a plant, a small water droplet probably due to air condensation was sometimes found inside the sachet after 48 h, even those without a planthopper. The volume of the droplet was usually less than a few mg. In the honeydew excretion experiment we could not distinguish air condensation from the planthopper excretion, and probably weighed a mixture of the two. When honeydew excreted by a planthopper was heavy enough, the influence of the droplet was negligible. But, the original data tended to overestimate the virulence of the population in cases in which most individuals of the population could not feed on the rice. To prevent this overestimation, we made the 3 mg subtraction from the original data indicated above. This procedure gives us a slight underestimation of the average quantity of the excretion in most cases, but a satisfactory estimation of relative excretion on resistant varieties compared to a susceptible variety.

Standard rice varieties used were TN1 (susceptible control), Mudgo (resistant gene; *Bph1*), IR26 (*Bph1*), ASD7 (*bph2*), Rath heenati (*Bph3*) and Babawee (*bph4*). The rice plants used for the experiment were basically 30- to 60-day-old plants in the tillering stage. Thirty-day-old ratoons were used in an experiment with the North Vietnamese population.

RESULTS

The mean amount of honeydew excreted for 48 h by a female adult on TN1 ranged from 23.6 to 61.8 mg (after 3 mg subtraction), and mainly depended on the populations and the stages of rice used for the experiments (Table 2). For standardization, we calculated an index of the excretion of each individual relative to the average excretion on TN1 in each experiment (=100) in each population. The mean of the indices of relative excretions (MRE) on the resistant varieties was used for the comparison of relative virulence among the populations or the varieties.

The MRE on the resistant varieties in the two replicated experiments attained almost similar values in all of the populations (including the Malaysian population, in which different wing-form adults were used in the two experiments). Therefore, the data of the two replicates were combined and were shown in Table 2.

The virulence on ASD7 differed greatly among the populations. The MRE ranged from 10.6 to 26.8 for the populations collected in Kyushu and the Red River Delta. This may indicate ASD7 was moderately resistant to these populations. On the other hand, the females from the Malaysian, Thai and Mekong Delta populations excreted almost equal amounts of honeydew when feeding on ASD7 as on TN1. The MRE ranged from 72.9 to 97.1, indicating that the resistance of ASD7 to BPH had entirely broken down in the tropical rice fields of the Indochina Peninsula.

The MRE on IR26 ranged from 32.8 to 51.6 for Japanese and Red River Delta populations, suggesting that IR26 was moderately susceptible. All tropical populations except the Malaysian one tended to be more capable of feeding on IR26. Mudgo, which has the same resistance gene (*Bph1*) as IR26, showed more resistance than IR26 for all populations.

Table 2. Means of the index of relative excretion (MRE) on six standard rice varieties in BPH populations collected in various places

Population	Insects used	Mean[c] on TN1 (mg)	MRE[a,b] on resistant varieties (resistance gene)					
			TN1 (none)	Mudgo (*Bph1*)	IR26 (*Bph1*)	ASD7 (*bph2*)	Rathu heenati (*Bph3*)	Babawee (*bph4*)
Kyushu A	44	23.6	100	3.2 a	32.8 ab	10.6 a	4.5 ab	6.9 ab
Kyushu B	51	30.7	100	13.9 ab	34.8 ab	15.6 a	3.5 b	4.6 ab
Red River Delta A	58	24.5	100	21.0 b	51.6 abc	26.8 a	2.8 ab	4.0 a
Red River Delta B	54	27.7	100	4.7 a	41.8 ab	13.6 a	0.5 a	2.8 a
Mekong Delta A	52	32.5	100	21.2 ab	59.7 bc	72.9 b	8.5 b	20.1 b
Mekong Delta B	51	32.3	100	19.1 ab	78.7 c	97.1 b	6.1 ab	9.3 ab
Thailand	53	26.5	100	11.1 a	58.1 bc	75.0 b	2.7 ab	3.4 ab
Malaysia[d]	53	61.8	100	4.3 a	20.0 a	75.3 b	0.5 a	6.4 ab

[a] Mean of the index of honeydew amount excreted by a female macropter on a resistant variety relative to the mean of the honeydew amount excreted on TN1 (=100).

[b] In a column, means followed by a letter are not significantly different at 5% level, according to the KRUSKAL-WALLIS and modified SCHEFFE method (SHIRAHATA, 1987).

[c] Mean amount of the honeydew excretion on TN1.

[d] Approximately half of the insects used were brachypters.

Table 3. Proportions (%) of adults[a] which excreted 10 mg or more for 48 h on the standard rice varieties in various BPH populations

Population	Insects tested[b]	Standard rice variety (resistant gene)					
		TN1 (none)	Mudgo (*Bph1*)	IR26 (*Bph1*)	ASD7 (*bph2*)	Ruth Heenati (*Bph3*)	Babawee (*bph4*)
Kyushu A	44	93.2	2.3	31.8	9.1	0	0
Kyushu B	51	94.1	13.7	41.2	15.7	0	2.0
Red River Delta A	58	93.1	22.4	44.8	29.8	1.7	1.7
Red River Delta B	54	92.5	3.7	46.3	11.8	0	2.0
Mekong Delta A	52	96.2	23.5	84.3	80.8	11.5	21.2
Mekong Delta B	51	98.0	22.4	84.3	88.0	10.0	6.1
Thailand	53	94.3	9.4	67.9	86.5	0	0
Malaysia	53	96.2	7.7	34.0	92.2	0	9.6

[a] Newly emerged macropterous adults were used for the experiment except for the Malaysian population in which approximately half of the planthoppers used were brachypters.

[b] Numbers of insects used were slightly different among varieties.

The MRE values on Rath Heenati and Babawee were very low, except in the Mekong Delta populations. This indicates that these varieties remained highly resistant in other areas.

Although MRE is a useful index for the comparison of relative virulence among populations, the proportion of planthoppers capable of certain levels of consumption may be a better index of biotype property for characterizing local BPH populations. Planthoppers on resistant varieties tended to be grouped into individuals that excreted little or no honeydew and those that excreted honeydew in amounts similar to that excreted by planthoppers on a susceptible variety. As a result, the MRE value did not show actual features of average individuals of the population. Other authors (SOGAWA, 1992 a; TAKAHASHI et al., 1994) used 10 mg of excretion as a threshold of feeding capability and virulence on rice. Therefore, Table 3 shows the proportion of adults that excreted 10 mg or more (subtraction of 3 mg was done after comparison with other reports) on the standard varieties.

The results obtained were quite similar to those obtained with analysis of MRE values. The proportion of females in the tropical Indochina population that was adapted for ASD7 was much greater than those of Japan and the Red River Delta (χ^2 test, $p<0.01$). The tendency was again recognized that the populations in the Mekong Delta and Thailand were more capable of feeding on IR26 than Japanese and Red River Delta populations (χ^2 test, $p<0.01$). Mekong Delta populations contained some planthoppers capable of feeding on either Rath Heenati or Babawee. The value on Babawee in the Malaysian population was also notable.

DISCUSSION

The biotypes of BPH populations and their shifts have been reported in East Asia and Indochina. In Japan, a gradual change from biotype 1 (capable of attacking

rice without any resistant gene) to biotype 2 (capable of attacking *Bph1*-resistant cultivars) in immigrants was observed during the period from 1987 to 1991 (SOGAWA, 1992 a, b). In Korea, biotype 1 was predominant but significant numbers of biotype 2 (ca. 25%) and biotype 3 (capable of attacking *bph2*-resistant cultivars; ca. 15%) were recorded in the population between 1985 and 1987 (PARK and SONG, 1988). In China, the BPH populations collected in 1987 and 1988 had low survival rates on IR26 and IR36, but the survival of the populations on IR26 collected in 1989 and 1990 showed similar values to that on TN1. This indicates a shift to biotype 2 (YU et al., 1991). In North Veitnam, the populations were biotype 2 (ICH, 1991) in 1990 (THUAT et al., 1992). In South Vietnam, *Bph1*-resistant varieties became susceptible from 1977, and in late 1986 and early 1987 a new biotype which attacks *bph2*-resistant varieties first appeared in the Mekong Delta (HUYNH, 1988). The populations collected in 1990 to 1991 were capable of attacking varieties with either *Bph1*, *bph2* or *bph4* genes (CHAU, 1992, 1993; THUAT et al., 1992). In Peninsular Malaysia, the population comprised a mixture of biotypes but the proportion of planthoppers capable of attacking either ASD7 or Mudgo was low in 1987–1990 (ITO et al., 1994).

The results obtained in this study basically coincided with information previously reported: a considerable proportion of individuals were capable of feeding on *Bph1*-resistant variety in Japanese and North Vietnamese populations, and the tropical populations had virulence to varieties with either the *Bph1* or *bph2* gene. At variance with previous work was the fact that virulence to ASD7 in the Malaysian population was much greater than that reported by ITO et al. (1994). However, Japanese populations and Red River Delta populations cannot be called biotype 2. Approximate 65% of planthoppers were not able to feed on IR26. Mudgo was even more resistant. An average of 12% were capable of feeding on ASD7. Probably, planthoppers which were able to feed on both IR26 and ASD7 (biotype 4; IRRI, 1974) were included in the population. In this sense, the classification of biotype based on gene-for-gene relationship proposed by IRRI (1974, 1975) cannot be applied to the population for identifying BPH biotype property. It is necessary to determine the proportion of planthoppers in the population which are able to attack each resistant variety.

The degree of resistance was quite different between IR26 and Mudgo, although both have the same *Bph1*-resistant gene. This was not surprising because it is commonly observed that varieties or cultivars with identical resistance genes show various degrees of resistance in field screening tests. Varietal resistance seems to be largely affected by minor genes as well as a major resistant gene.

BPH populations collected from tropical Indochina Peninsula were clearly distinguished from the populations of Japan and subtropical North Vietnam, on the basis of virulence to ASD7. In addition, biotype compositions in the tropics were somewhat different among the local populations. The difference of biotype compositions in the tropics can probably be attributed to the history of resistant varieties in the area.

However, a quick shift in BPH biotype in the tropical rice fields was noted in cases that lacked significant changes in coverage of resistant varieties. In Muda, Malaysia, virulence to ASD7 appeared to greatly increase during the course of the present study, in comparison to the results obtained a couple of years ago by ITO et al. (1994). The resistant varieties adopted there did not appreciably change during this period, and included only a few *bph2*-varieties (mostly IR42) which account for ca. 25% of the rice area. This quick change of the biotype composition in the population may

indicate migration across country borders in the tropics. HABIBUDDIN (1989) and ITO et al. (1994) also pointed out the possibility of a BPH invasion from Sumatra, according to the incomprehensible appearance of a new biotype on the Peninsula.

The biotype composition of Japanese BPH bore close resemblance to those of the populations in the Red River Delta, and although further studies should be carried out, this finding may support the hypothesis that North Vietnam is one of the source areas of Japanese planthoppers.

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