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Brown Planthopper *Nilaparvata lugens* STÅL (Homoptera: Delphacidae) Biotypes Capable of Attacking Resistant Rice Varieties in Malaysia

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Biotypes of brown planthopper populations collected in and around the Muda area in Peninsular Malaysia in 1989 and 1990 were examined by comparing the amount of honeydew excreted by the female adults on 5 standard rice varieties: Mudgo (which has the *Bph 1* gene for resistance to the brown planthopper), ASD7 (*bph 2*), Rathu Heenati (*Bph 3*), Babawee (*bph 4*) and TN1 (no resistance genes). Most populations from the Muda area showed similar biotypical properties regardless of the collection sites or crop seasons. Among the 4 resistant varieties used, high mortality was recorded on Rathu Heenati and Babawee. A relatively larger amount of honeydew was discharged on ASD7; this was followed by Mudgo. Little honeydew excretion was recorded on Rathu Heenati and Babawee. A similar trend was also observed in most populations collected from other sites on the west and east coasts of Peninsular Malaysia. Two explanations are considered for this phenomenon, i.e., these biotypes had developed in Malaysia or immigrated from Sumatra, Indonesia.

Key words: *Nilaparvata lugens*, biotype, honeydew, resistant variety, Malaysia

INTRODUCTION

The development of host plant resistance is one of the most practical measures to solve insect pest problems in the integrated pest management strategy (OKA and BAHAGIAWATI, 1984; SAXENA and BARRION, 1987). To use insect-resistant rice varieties is an ideal and attractive method for farmers to control the brown planthopper (abbr. BPH) *Nilaparvata lugens* STÅL, one of the serious insect pests of rice, because it is simple and inexpensive in comparison with other methods of control. A total of 9 BPH-resistance genes (4 dominant and 5 recessive) have been identified thus far in rice (KANEDA, 1988). Many BPH-resistant varieties have been bred and distributed in South and Southeast Asia, mainly by International Rice Research Institute.

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However, some of these varieties lost their resistance within a few years after introduction due to the development of virulent BPH biotypes, or never showed field resistance at all. Such cases were reported in the Philippines, Vietnam, Indonesia and the Solomon Islands (Khush, 1979). This is a serious problem for the use or distribution of resistant varieties, because it takes a long time (several years or more) to breed a new resistant variety. It is, therefore, important to know the biotype of the local BPH before the introduction of new resistant varieties.

In the Muda irrigation scheme on the west coast of Peninsular Malaysia, non-resistant rice varieties have been cultivated in the greater part of the fields. A rice variety 'IR42' is planted in about 20% of the fields. Introduced in 1983 to control tungro disease, this variety is resistant to the green leafhopper *Nephotettix virescens* Distant, a vector of the tungro virus. In addition, this variety has a BPH-resistance gene, *bph 2*. There have not yet been any hopperburns in the fields planted with IR42 in the Muda area.

This study aims to identify the biotypes of BPH collected in and around the Muda area by comparing the amount of honeydew excreted by adult females that feed on several resistant rice varieties.

MATERIALS AND METHODS

Insects. A total of 16 BPH populations was collected from various rice fields in Malaysia from July, 1989 through July, 1990; 9 populations from the Muda area, 4 from other areas on the west coast, 2 from the east coast and 1 from Sabah, Borneo (Fig. 1, Table 1). Each population was kept in a rearing cage with rice seedlings of a susceptible variety, 'TN1'. Female adults from the offspring generation were used

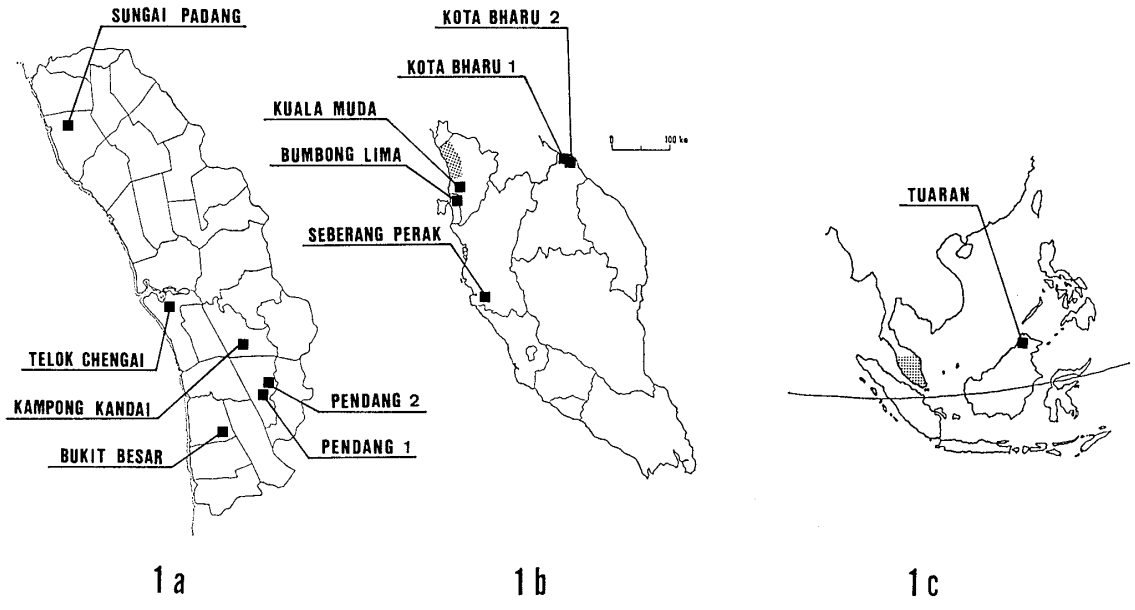

Fig. 1. Localities where BPH populations were collected. The area corresponding to Peninsular Malaysia (c) was magnified and placed in b. The Muda area in b (shaded part) was magnified and shown in a.

Table 1. BPH populations used for the biotype experiment

Collection site	Population		Collection date	Tested generation (collected gen.=0)
Muda area	Sungai Padang	8907	12 Jul. 1989	1
	Telok Chengai	8907	26 Jul. 1989	1
	Pendang 1	8907	31 Jul. 1989	1
	Pendang 2	8907	31 Jul. 1989	1
	Bukit Besar	8907	12 Jul. 1989	1
	Telok Chengai	9001	10 Jan. 1990	4
	Kampung Kandai	9002	12 Feb. 1990	2
	Bukit Besar	9006	26 Jun. 1990	0
	Telok Chengai	9007	26 Jul. 1990	3
Outside Muda (west coast of Pen. Malaysia)	Kuala Muda	8903	2 Aug. 1989	1
	Seberang Perak	8912	8 Dec. 1989	1
	Bumbong Lima	—[a]	?	?
	Seberang Perak	9007	5 Jul. 1990	4
East coast of Pen. Malaysia	Kota Bharu 1	8908	12 Aug. 1989	1
	Kota Bharu 2	8908	12 Aug. 1989	1
Sabah	Tuaran	9001	? Jan. 1990	?[b]

[a] A stock culture population at MARDI Rice Research Center maintained for 10 years or more. Tested on 24 June 1990.
[b] Tested on 16 May 1990.

for the test (Table 1). The Bumbong Lima population was derived from the stock culture maintained at the MARDI Rice Research Center, which originated from a population collected near the Center and had been maintained on TN1 for more than 10 years. The original Tuaran population was collected in January, 1990 and has been maintained on the susceptible variety 'Bahagia' at the Agriculture Research Center in Tuaran, Sabah. The first generation adults developed on TN1 were used for the test.

Bioassay of BPH biotypes. It was postulated that the honeydew excretion by a BPH was proportional to the amount of the sap sucked by it from the rice plant. Thus, the quantity of honeydew excreted by an insect is considered to be an index of the amount of sap sucked on each variety (SOGAWA and PATHAK, 1970; SOGAWA, 1981 a), that is, a biotype index.

The rice varieties used to examine biotype were 'Mudgo' (with the *Bph 1* gene for resistance to BPH), 'ASD7' (*bph 2*), 'Rathu Heenati' (*Bph 3*), 'Babawee' (*bph 4*) and 'TN1' (no resistance genes). The seedlings of these varieties were transplanted in small plastic pots 10 days after sowing and 40- to 50-day-old seedlings were used for the experiment. A transparent plastic feeding chamber with a filter paper at the bottom was placed at the basal part of the plant. Three- to seven-day-old females were individually introduced into each feeding chamber and was allowed to feed on the plant for 2 days. Either brachypterous or macropterous females were used in each test. The test was carried out at room temperature (25–30°C). The amount of honeydew collected on the filter paper placed at the bottom of the chamber was visualized by the ninhydrin treatment method (SOGAWA, 1970) or the bromocresol green treatment method (HEINRICHS et al., 1985).

In the ninhydrin treatment method, the filter papers were removed from the chamber and sprayed with a solution of 0.01% ninhydrin in acetone, and then dried in an oven for 30 min at 70°C. Purple spots appeared due to the amino acids contained in the honeydew. In the bromocresol green treatment method, the filter paper was pretreated with a solution of 0.2% bromocresol green in ethanol and air-dried. Since bromocresol green is a pH indicator, blue spots appear on the yellow-orange filter paper immediately after honeydew drops on it. The bromocresol green method was preferred in this study because the purple honeydew spots fade rapidly in the ninhydrin method (Heinrichs et al., 1985).

A tracing section paper was placed over the treated filter paper and the outline of honeydew patches were traced. The area of the patches was measured in mm^2 and was considered to reflect the amount of honeydew or the sap ingested by the female on each variety (Heinrichs et al., 1985).

Twelve to thirty-six females were used for each variety. All the insects, including ones which died during the test, were considered for the analysis. This study was carried out at the MARDI Alor Setar Station, Malaysia.

RESULTS

The patch area of honeydew excreted by the Telok Chengai population collected on 26 July 1989 (abbr. T. Chengai 8907), on the 5 different varieties is shown in Fig. 2 as an example. On the susceptible variety TN1, the area corresponded to 0–330.5 mm^2 (average=198.9 mm^2) and 2 insects died during the test. Considerable individual variations in the amount of excretion were observed on TN1 as well as on the resistant varieties. On ASD7, the average area of patches was 95.6 mm^2, and that on Mudgo was 52.5 mm^2. Some females on ASD7 and Mudgo excreted as much honeydew as those on TN1. However, the excretion on Rathu Heenati and Babawee was much smaller than that on ASD7 or Mudgo. The average was 6.5 mm^2 on Rathu Heenati and 22.4 mm^2 on Babawee. Many insects died on these 2 varieties during the test; 14 and 12 out of 18 insects tested on Rathu Heenati and Babawee, respectively.

The percentage of insects that died during the test on each variety is shown in Table 2. The mortality was high on Rathu Heenati and Babawee, followed by Mudgo or ASD7 in most populations. There were some populations (T. Chengai 9001, K. Kandai 9002 and K. Bharu 1 8908) in which the mortality on Rathu Heenati and Babawee was low. In these cases, the mortality on Mudgo and ASD7 was also low. Although the mortality on resistant varieties is a convenient biotype index, our testing period (2 days in this experiment) was short and possibly insufficient for examining in detail the biotypical properties of the populations.

The amount of excreted honeydew differed very much among the tested populations, even on the susceptible variety TN1. This may be due to the fact that feeding activity is affected by the seedling and/or the insect conditions. Therefore, we compared the honeydew excretions of the populations in 2 ways. First, the females that excreted honeydew with patches of areas of 50 mm^2 or more were tentatively classified as individuals capable of feeding on that variety. The percentage of such females was compared among the populations. Secondly, we standardized the data: we selected half replicates (half of the tested insects) with larger amounts of excretions from each variety in each population (for instance, when 18 females were tested, the 9 females with the

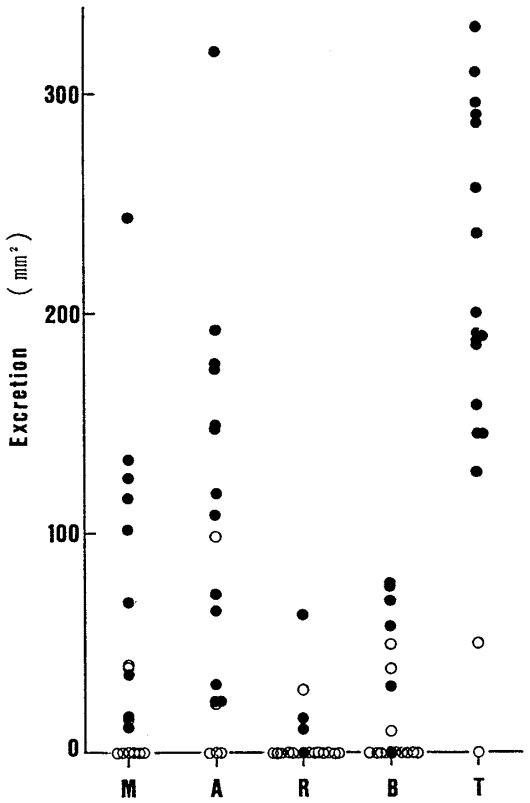

Fig. 2. Amount of honeydew excreted by females of the Telok Chengai population collected on 26 July 1989. Each circle represents the amount of honeydew excreted by an individual insect. Black and white circles represent insects that survived and died during the test, respectively. M: Mudgo, A: ASD7, R: Rathu Heenati, B: Babawee, T: TN1.

highest excretions were selected), and the average excretion on TN1 of these selected insects in each population was rated as 100. The relative excretion of each insect was then calculated. The means of the rate (excretion index) in a population was used in a comparison of the amount of sap sucked among the varieties and the populations.

The percentage of females in which the patch area of excreted honeydew corresponded to 50 mm^2 or more is shown in Table 3. More than 65% of the females in each population were classified as individuals that were able to feed on TN1. Although a low percentage of females ($<30\%$) were able to feed on Rathu Heenati and Babawee, a fairly large number of females were able to feed on ASD7 in most of the populations. The percentage on ASD7 was lowest in the stock culture population (B. Lima) and the population collected from Tuaran. On Mudgo, the percentage varied among the populations.

The excretion indices for the populations tested on different varieties are presented in Table 4. Among the 4 resistant varieties, insects generally showed larger levels of excretion on ASD7. This level was followed by that of Mudgo, while the excretions on Rathu Heenati and Babawee were small. The populations of K. Kandai 9002 (excretion index 78.1), S. Perak 9007 (55.6), B. Besar 8907 (54.8) and B. Besar 9006

Table 2. Mortality (%)[a] of BPH populations on 5 rice varieties

Population		Rice variety ⟨resistance gene⟩				
		Mudgo ⟨*Bph 1*⟩	ASD7 ⟨*bph 2*⟩	Rathu Heenati ⟨*Bph 3*⟩	Babawee ⟨*bph 4*⟩	TN1 ⟨none⟩
S. Padang	8907	58.3 [50.0]	33.3 [25.0]	81.8 [73.5]	75.0 [66.7]	8.3
T. Chengai	8907	50.0 [38.9]	33.3 [22.2]	77.8 [66.7]	66.7 [55.6]	11.1
Pendang 1	8907	29.4 [12.7]	27.8 [11.1]	44.4 [27.8]	38.9 [22.2]	16.7
Pendang 2	8907	35.3 [24.2]	38.9 [27.8]	66.7 [55.6]	83.3 [72.2]	11.1
B. Besar	8907	33.3 [25.0]	33.3 [25.0]	75.0 [66.7]	16.6 [8.3]	8.3
T. Chengai	9001	5.6 [−11.1]	11.1 [−5.6]	22.2 [5.6]	5.6 [−11.1]	16.7
K. Kandai	9002	0 [0]	6.3 [6.3]	5.6 [5.6]	5.6 [5.6]	0
B. Besar	9006	27.8 [21.5]	11.1 [4.8]	50.0 [43.3]	55.6 [49.3]	6.3
T. Chengai	9007	36.1 [36.1]	16.7 [16.7]	44.4 [44.4]	36.1 [36.1]	0
K. Muda	8908	29.4 [18.3]	5.6 [−5.6]	55.6 [44.4]	77.8 [66.7]	11.1
S. Perak	8912	38.9 [27.8]	33.3 [22.2]	70.6 [59.5]	72.2 [61.1]	11.1
S. Perak	9007	46.7 [19.7]	26.7 [−0.3]	32.3 [5.3]	34.5 [7.6]	26.9
B. Lima	—	32.4 [7.4]	38.9 [13.9]	58.3 [33.3]	61.1 [36.1]	25.0
K. Bharu 1	8908	0 [−5.6]	0 [−5.6]	22.2 [16.7]	5.6 [0]	5.6
K. Bharu 2	8908	61.1 [61.1]	38.9 [38.9]	77.8 [77.8]	61.1 [61.1]	0
Tuaran	9001	41.7 [33.3]	37.1 [28.8]	61.1 [52.8]	47.2 [38.9]	8.3

[a] Figures in brackets show corrected mortality (=mortality on resistant variety − mortality on TN1).

Table 3. Percentage of BPH females which excreted 50 mm² or more honeydew for 2 days on 5 rice varieties

Population		Rice variety ⟨resistance gene⟩				
		Mudgo ⟨*Bph 1*⟩	ASD7 ⟨*bph 2*⟩	Rathu Heenati ⟨*Bph 3*⟩	Babawee ⟨*bph 4*⟩	TN1 ⟨none⟩
S. Padang	8907	25.0	25.0	0	0	91.7
T. Chengai	8907	33.3	61.1	5.6	22.2	94.4
Pendang 1	8907	35.3	44.4	5.6	5.6	100
Pendang 2	8907	47.1	50.0	11.1	5.6	88.9
B. Besar	8907	58.3	33.3	16.7	0	100
T. Chengai	9001	5.6	33.3	0	0	66.7
K. Kandai	9002	80.0	62.5	22.2	27.8	100
B. Besar	9006	61.1	66.7	5.6	5.6	100
T. Chengai	9007	25.0	55.6	8.3	2.8	100
K. Muda	8908	5.9	59.6	5.6	5.6	83.3
S. Perak	8912	11.1	27.8	5.9	0	66.7
S. Perak	9007	23.3	43.3	3.2	3.4	65.3
B. Lima	—	29.4	11.1	0	0	80.6
K. Bharu 1	8908	38.9	55.6	0	5.6	94.4
K. Bharu 2	8908	11.1	44.4	0	11.1	88.9
Tuaran	9001	13.9	20.0	0	0	86.1

Table 4. Excretion index ('TN1'=100) of BPH populations on 5 rice varieties

Population		No. tested	Rice variety ⟨resistance gene⟩				
			Mudgo ⟨*Bph 1*⟩	ASD7 ⟨*bph 2*⟩	Rathu Heenati ⟨*Bph 3*⟩	Babawee ⟨*bph 4*⟩	TN1 ⟨none⟩
S. Padang	8907	12	40.0	45.2	3.7	16.4	100
T. Chengai	8907	18	37.7	62.1	4.9	16.8	100
Pendang 1	8907	18	33.0	29.6	8.5	6.0	100
Pendang 2	8907	18	42.7	51.3	11.8	8.8	100
B. Besar	8907	12	54.8	70.2	18.6	20.4	100
T. Chengai	9001	18	22.6	81.6	4.9	1.2	100
K. Kandai	9002	18	78.1	109.5	30.8	33.2	100
B. Besar	9006	18	54.2	50.7	8.6	5.6	100
T. Chengai	9007	36	30.8	65.3	11.0	13.9	100
K. Muda	8908	18	24.7	60.8	14.5	10.0	100
S. Perak	8912	18	45.6	55.1	14.7	8.0	100
S. Perak	9007	36	55.6	62.1	18.3	13.8	100
B. Lima	—	36	30.6	23.3	2.7	1.7	100
K. Bharu 1	8908	18	41.6	53.3	2.7	7.0	100
K. Bharu 2	8908	18	13.8	35.9	4.2	8.4	100
Tuaran	9001	36	22.4	24.5	1.4	2.6	100

(54.2) showed relatively higher excretion indices on Mudgo. On ASD7, 11 populations showed the excretion indices above 50, i.e., K. Kandai 9002 (109.5), T. Chengai 9001 (81.6), B. Besar 8907 (70.2), T. Chengai 9007 (65.3), T. Chengai 8907 (62.1), K. Muda 8908 (60.8), S. Perak 9007 (62.1), S. Perak 8912 (55.1), K. Bharu 1 8908 (53.3), Pendang 2 8907 (51.3) and B. Besar 9006 (50.7). In contrast, all the populations tested on Rathu Heenati and Babawee showed very low excretion indices, except for the K. Kandai 9002 populations in which the indices were 30.8 on Rathu Heenati and 33.2 on Babawee.

Although the K. Kandai 9002 population showed a higher level of excretion of honeydew on all 4 of the resistant varieties, the reason was unknown. Populations of B. Lima and Tuaran excreted only a small amount of honeydew on ASD7: the excretion index was 23.3 for B. Lima and 24.5 for Tuaran, the smallest among the tested populations on ASD7.

DISCUSSION

A new BPH biotype was first recognized in 1975. A BPH-resistant variety with the *Bph 1* gene, IR26, became susceptible in a rice field in the Philippines (Feuer, 1976; Sogawa, 1982). A similar phenomenon was reported soon afterwards in the Mekong Delta of Vietnam in 1977 (Nguyen, 1977). IR42 and IR36 (both varieties have the *bph 2* gene) were then widely planted in Southeast Asia. However, another new biotype capable of breaking down the resistance of the *Bph 1* and *bph 2* genes has been reported in some countries in recent years, such as in the Philippines (Medrano and Heinrichs, 1985), Indonesia (Oka and Bahagiawati, 1984) and Vietnam (Luong,

1990). These biotypes were identified on the basis of the seedbox screening test (see Heinrichs et al., 1985).

Habibuddin (1989) reported that resistant varieties with the *Bph 1*, *bph 2*, *Bph 3* or *bph 4* gene still maintained resistance on the east coast of Peninsular Malaysia, while the BPH populations on the west coast contained some biotypes capable of attacking varieties with the *Bph 1* or *bph 2* gene. He had collected BPH populations in 1986 and identified biotypes by using the seedbox screening test.

Our data indicated that most of the populations in the Muda area were of generally similar biotypes regardless of the collection sites or crop seasons. The mortality was lower on ASD7 and Mudgo than that on Rathu Heenati and Babawee (Table 2). The largest honeydew excretion was observed on ASD7 among the 4 resistant varieties followed by Mudgo, while excretions were small on Rathu Heenati and Babawee (Tables 3, 4). This trend was also observed in most of the populations collected from the west and east coasts of Peninsular Malaysia in 1989 and 1990. However, the stock-culture population (B. Lima) and the Tuaran populations seem to excrete a small amount of honeydew on ASD7. They are likely to be different from other populations, but further investigations are necessary.

It is notable that most of the populations in Malaysia contained biotypes capable of attacking ASD7 or Mudgo, while very few insects were able to attack Rathu Heenati or Babawee. IR42 (which possesses the *bph 2* gene as does ASD7) was introduced into the Muda area in 1983 and has occupied about 20% of the planted area in recent years. Exceptions were the main season (=2nd) crop of 1984 (41.5%) and the off-season (=1st) crop of 1985 (31.5%) (Fig. 3). 'MR77' (='Seberang,' with the same *Bph 1* gene as Mudgo) was introduced into the Muda area in 1984. Percentages of

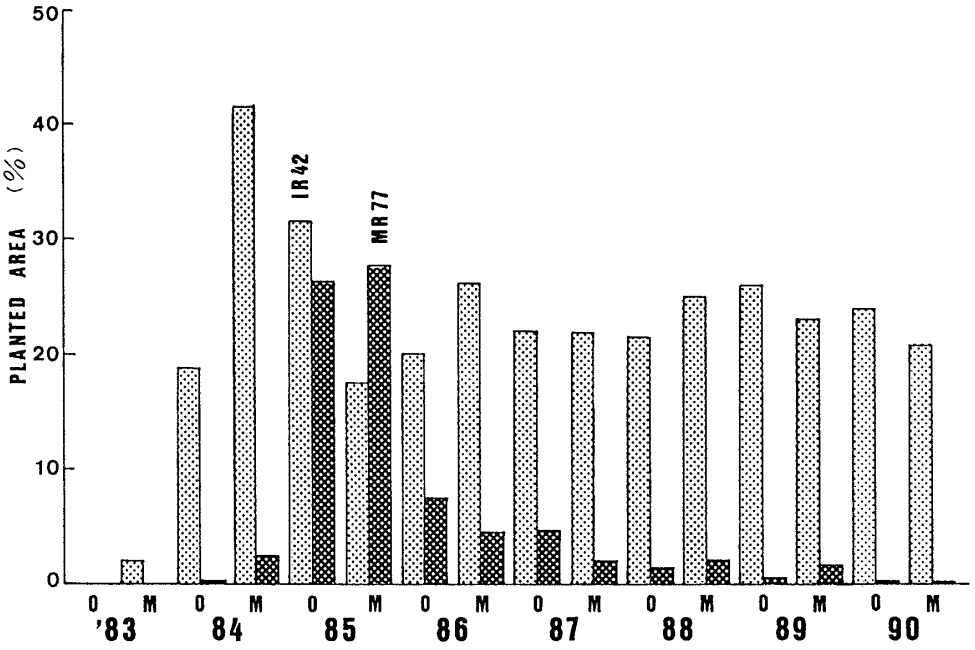

Fig. 3. Annual changes in the share of BPH-resistant varieties planted in the Muda area. Only 2 resistant varieties, IR42 (with the *bph 2* gene) and MR77 (*Bph 1*), have been cultivated thus far. O: off-season crop (=first crop), M: main season crop (=second crop).

cover were 26.2% and 27.7% of the planted area in the off- and main seasons of 1985, respectively. However, this has decreased to less than 5% in recent years (Fig. 3), because another high-yielding variety 'MR84' (lacking BPH-resistant genes) was released. No other BPH-resistant varieties have been cultivated in this area and no BPH-resistant varieties have been widely introduced into other rice-growing regions in Malaysia.

One possible reason for the occurrence of biotypes capable of attacking ASD7 or Mudgo is that the biotypes had been selected in the Muda area. However it should be emphasized that the BPH had been able to choose and multiply on susceptible varieties such as MR84 that occupied about 60% of the planted fields in the Muda area for the last several years. Also there have been no reports of hopperburn in the fields planted with IR42 or MR77. These facts suggest that outbreaks of these biotypes did not occur. Laboratory experiments showed that some characteristics of biotype 2 (*Bph 1*-attacking biotype) and biotype 3 (*bph 2*-attacking biotype) were entirely lost or diluted in hybridization with biotype 1 (attacking only susceptible varieties) (SOGAWA, 1981 b). ITO and KISIMOTO (1981) also reported that each F_1 progeny in crosses between Mudgo-infesting biotype and biotype 1 or between ASD7-infesting biotype and biotype 1 was unable to attack Mudgo or ASD7. Therefore, it should take a considerable time for the development of a new biotype if the planted area of a resistant variety is small. Accordingly, this possibility that the biotypes had developed in the Muda area seems to be weak, though it is undeniable.

Another possible reason is the immigration from Sumatra (HABIBUDDIN, 1989). There are several facts supporting this assumption: 1) the biotypes capable of attacking resistant varieties which have the *Bph 1* or *bph 2* genes were reported from North Sumatra (OKA and BAHAGIAWATI, 1984); 2) the southwest monsoon (from Sumatra to Peninsular Malaysia) predominates from May through October; 3) since BPH can migrate from mainland China to Japan (the distance is at least 1,000 km) in the monsoon (KISIMOTO and DYCK, 1976), they can easily cover the distances between Sumatra and the west coast of Peninsular Malaysia, which are as little as 100 km. Although this hypothesis seems to be more plausible than the former, there is no direct evidence that BPH immigrate from Sumatra.

This study indicates that BPH populations in Peninsular Malaysia are the mixture of the biotypes: a considerable proportion of insects are able to feed on the varieties which have the *Bph 1* and/or *bph 2* genes. No large fluctuation was observed in the biotypes during the 2 years. It is, however, necessary to monitor the change of biotypes in the BPH populations regularly.

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