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Biotypic Variations in the Brown Planthopper, *Nilaparvata lugens* (Homoptera : Delphacidae) at the IRRI, the Philippines

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The biological nature of three biotypes of the brown planthopper, *Nilaparvata lugens*, which possess differential abilities to infest resistant rice varieties, was comparatively studied with respect to their feeding effect on rice seedling growth, host preference response, honeydew excretion, embryonic and nymphal development, and fecundity. The three biotypes were most clearly distinguished from one another on the basis of their average honeydew excretion and the number of progeny produced on rice varieties with different major genes for resistance to this insect in spite of large quantitative variabilities in these physiological traits within each biotype population. In addition, biotype 3 differed significantly from the others, particularly from biotype 1, in its feeding effect on seedling growth, host preference, and nymphal development on resistant varieties. Biotype 3 was also found to be less fecund than others even on a susceptible variety.

INTRODUCTION

With the spread of high-yield rice varieties and of intensive cultivation, the brown planthopper, *Nilaparvata lugens*, has become the most destructive rice pest throughout tropical Asia since about 1970 (DYCK and THOMAS, 1979). The existence of host resistance-breaking biotypes has further complicated the control strategies for this rice pest through genetic manipulation in rice varieties (IRRI, 1976). A biotype of the brown planthopper is generally referred to as a population which has a specific ability or inability to survive on and infest rice varieties with specific genes for resistance to this insect. The population which cannot infest any varieties with resistance genes is called biotype 1, while those populations infesting resistant varieties carrying *Bph 1* and *bph 2* genes are described as biotypes 2 and 3, respectively (IRRI, 1976). These sympatric biotypes have identical morphological characters, and no reproductive barriers exist between them (LIQUIDO, 1978).

The present experiments were conducted to characterize and differentiate the brown planthopper biotypes by their biological reactions to susceptible and resistant rice varieties.

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MATERIALS AND METHODS

Brown planthopper biotypes 1, 2, and 3, maintained as isolated inbred populations at IRRI, were used. Biotype 1 was kept on susceptible Taichung native 1 (TN 1) since 1965. Biotypes 2 and 3 were developed from natural populations by forced breeding on the resistant varieties Mudgo with *Bph 1* and ASD 7 with *bph 2* genes, respectively (IRRI, 1976).

Three rice varieties were utilized, IR 24 with no resistance gene, IR 26 with the *Bph 1* gene, and IR 40 with the *bph 2* gene. In some experiments, another susceptible variety TN 1 was used instead of IR 24.

The following five biological responses of the biotypes were compared: (1) effect of feeding on seedling growth, (2) host preference, (3) honeydew excretion, (4) embryonic and nymphal development, and (5) reproduction.

Effect of feeding on seedling growth. Pregerminated rice seeds were sown one by one in test tubes (1.5×15 cm) containing about 5 ml of submerged soil. Two days after sowing, newly emerged female adults of each biotype were introduced individually into each test tube, and allowed to feed on the seedlings for 2 days at room temperature. Thereafter the length of the rice seedlings was measured.

Host preference. About one-month-old resistant and susceptible varieties were planted side by side in a clay pot (18 cm in diameter). About 20–30 female adults of each biotype were placed on a resistant variety at the beginning of the experiment. Then the migration of the insects from the resistant variety to a susceptible one was observed periodically.

Honeydew excretion. Twenty female adults of each biotype were confined individually on the basal portion of approximately one-month-old rice plants with an air-tight envelope made of parafilm (3×3 cm), and allowed to suck on the leaf sheath for a day. The amount of honeydew excreted was measured by weighing the parafilm envelope before and after removing the honeydew discharged in it with a Mettler microbalance. Experiments were conducted in an air-conditioned insectary at 28°C.

Embryonic and nymphal development. Two to four hundreds eggs deposited by each biotype were dissected out from the leaf sheaths of TN 1 plants 2 days after oviposition, placed on a small piece of moistened black cloth in a petri dish, and kept in an incubator adjusted to 28°C. The number of eggs hatched was recorded daily to compare the egg periods among the biotypes.

To examine the nymphal development, 20 newly emerged lst instar nymphs of each biotype were transferred individually to test tubes (1.5×15 cm) with a root-washed one-week-old rice seedlings and a small volume of tap water. The test tubes were then closed with a cotton plug, and kept in a cabinet at a constant temperature of 28°C. Nymphal growth and mortality were recorded every day until all the nymphs emerged to adults or died. Seedlings were replaced 2–3 times during the experiments.

Reproduction. Each single pair of newly emerged brachypterous males and females of the three biotypes was placed on approximately one-month-old rice plants in cylindrical plastic cages. After 25 days, the total number of progeny produced by each pair was counted. Twenty pairs for each biotype, and 10 pairs of inter-biotypic mating between biotypes 1 and 3 were tested. The reproductive potentials of the three biotypes on IR 24 were also compared by small paddy plot experiments, where 3 separate paddy plots of 2×2 m covered with a 3×3×2 m net-house were used. IR 24 seedlings aged 25 days were transplanted at 20 cm intervals on July 5, 1979. Fifty macropterous males and females of each biotype were released in each plot on July 25. Each 10 healthy hills were randomly sampled in a plot, and the number of insects on those 10 hills were counted on September 22, when the biotype 1 and 2 plots suffered partial hopperburn.

RESULTS

Effect of feeding on seedling growth

Both biotypes 1 and 2 caused a 40–50% reduction in seedling growth of IR 24 and IR 40. Their feeding effects were no different even on the seedlings of IR 26, which is resistant to the former, but susceptible to the latter. Biotype 3 prevented the seedling growth of IR 26 and IR 40 (causing about 60–65% reduction in seedling height) more severely than biotypes 1 and 2 (25–50% reduction), but the feeding effect on IR 24 seedlings was smaller (30% reduction) than that of biotypes 1 and 2 (40% reduction). There was no significant correlation between the ability of each biotype to infest resistant varieties and the short-term feeding effect on seedling growth of the respective resistant varieties (Table 1).

Table 1. HEIGHT (mm) OF RICE SEEDLINGS INFESTED 2 DAYS AFTER GERMINATION BY A SINGLE FEMALE ADULT OF EACH BIOTYPE FOR 2 DAYS[a]

Biotype	IR 24	IR 26	IR 40
1	53.7 c	57.4 b	31.2 b
2	53.8 c	57.6 b	34.3 b
3	63.9 b	30.5 c	22.0 c
Control	92.2 a	75.4 a	62.7 a

[a] Values followed by the same letter are not significantly different at the 5% level according to DMRT.

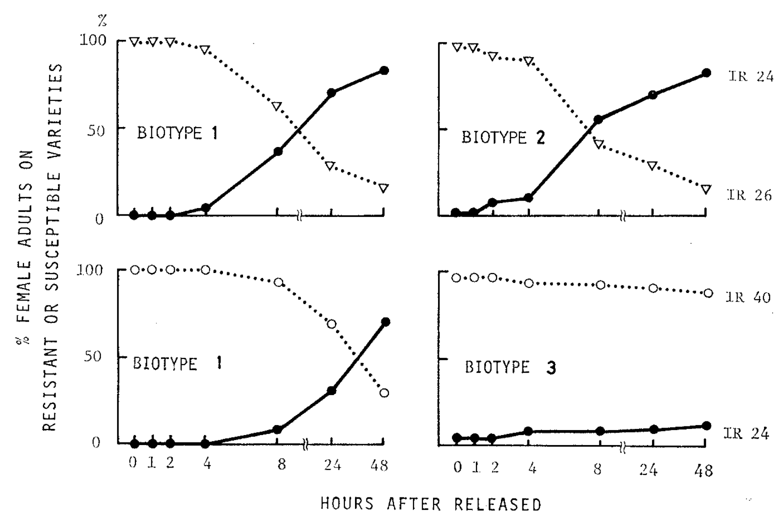

Fig. 1. Host preference responses of biotypes 1, 2 and 3.

Host preference

Under free-choice conditions, female adults of biotype 2 behaved similarly to biotype 1 in avoiding settling on IR 26. The differences in the host preference respones to IR 40 between biotypes 1 and 3 were conspicuous. Most of the females of biotype 1 did not settle on IR 40, and eventually moved to the susceptible IR 24. On the contrary, those of biotype 3 settled on IR 40 as shown in Fig. 1.

Honeydew excretion

There was a positive correlation between the amount of honeydew excreted and the ability of each biotype to infest resistant varieties (Table 2). Female adults of the three biotypes excreted each as much as 40–50 mg/day on TN 1 plants. On IR 26, biotype 2 females excreted nearly 30 mg/day while biotype 1 and 3 females excreted less than 10 mg of honeydew per day. Likewise, biotype 3 discharged about twice as much honeydew on IR 40 as did biotypes 1 and 2. However, a wide range of individual variation in daily honeydew excretion was observed within each biotype population, and the individual variations overlapped considerably among the biotypes.

Table 2. Average Weight of Honeydew Excreted by Female Adults of Each Biotype on Susceptible and Resistant Rice Varieties

Biotype	Variety	No. of insects observed	Average weight of honeydew	
			Actual value mg/insect	Transformed value[a] (±S.E.)
1	TN 1	49	49.1	1.51±0.06 a
	IR 26	27	6.5	0.73±0.05 c
	IR 40	16	16.7	1.06±0.09 b
2	TN 1	19	37.2	1.36±0.11 a
	IR 26	38	29.1	1.26±0.07 a
	IR 40	14	16.1	0.96±0.12 b
3	TN 1	20	46.0	1.56±0.09 a
	IR 26	15	9.7	0.68±0.12 c
	IR 40	36	31.2	1.31±0.08 b

[a] Transformed to $\log_{10}$ scale. Values followed by a common letter are not significantly different at the 5% level according to DMRT.

Embryonic and nymphal development

About 33–45% of the eggs hatched at the 7th day after oviposition. The hatching period lasted one week, and finally 80–85% of the eggs hatched by the 14th day after oviposition. There was no difference in both egg period or hatchability among the biotypes.

On the TN 1 seedlings, first instar nymphs of the three biotypes developed into adults within 12–14 days, and their growth was well synchronized. There was no significant difference in nymphal duration and mortality, or in the weight of newly emerged female adults. On the IR 26 and IR 40 seedlings, biotype 3 nymphs showed the best growth among the biotypes, although the total nymphal duration was slightly prolonged (13–16 days), and adults emerged on those varieties were smaller than those on TN 1. Nymphal mortality of biotypes 1 and 2 was higher on IR 26 and IR 40, particularly on the former, than that of biotype 3. The nymphal period of biotypes 1 and 2 on IR 26 and IR 40 varied greatly, ranging from 14 to 28 days. Such growth retardation and mortality occurred usually in the later stages of nymphal development. Biotype 1 and 2 nymphs emerged as significantly smaller adults on the resistant varieties. The nymphal growth response of biotypes 1 and 2 were not significantly different on IR 26. On the other hand, differences in nymphal

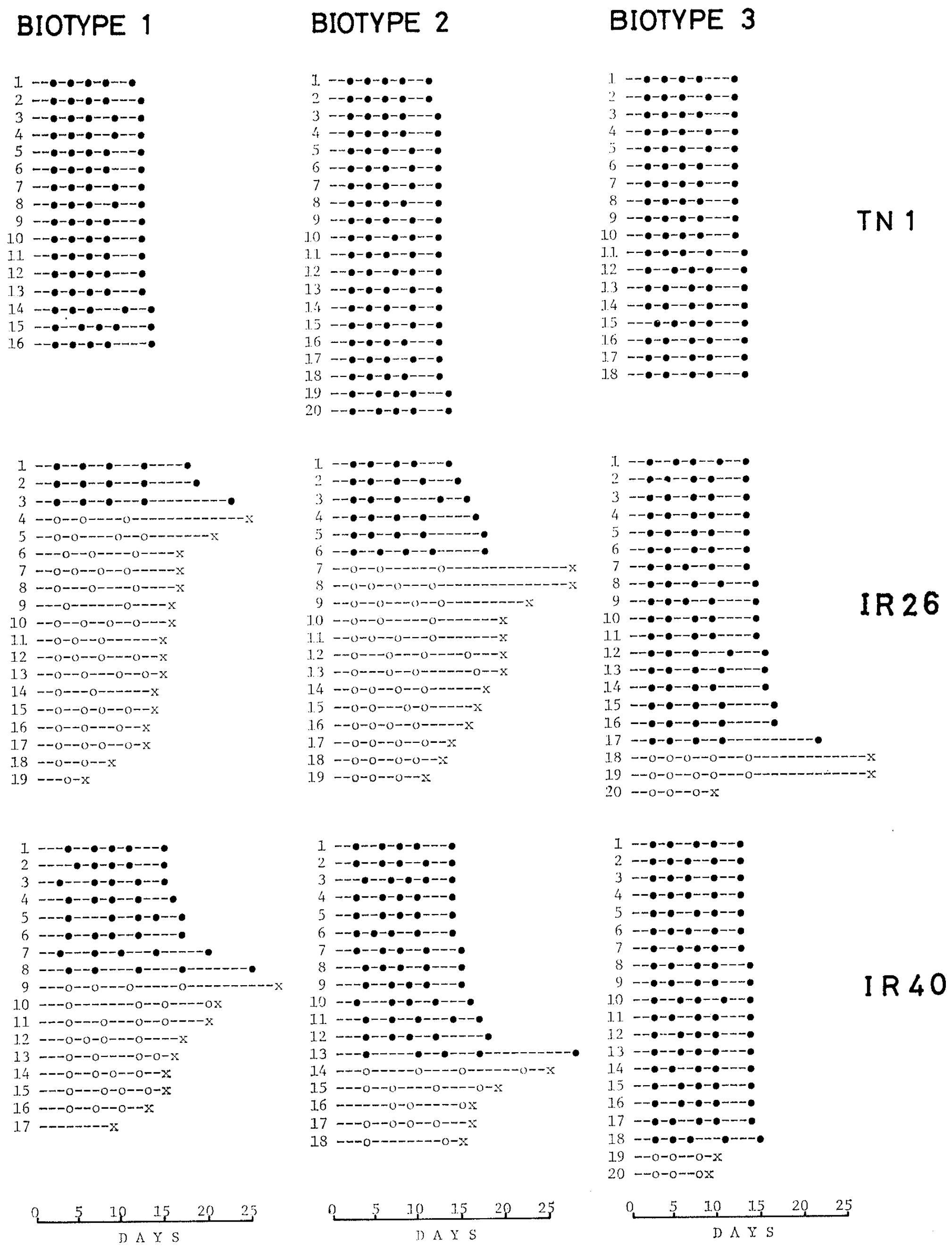

Fig. 2. Individual records of nymphal development of biotypes 1, 2 and 3 on susceptible and resistant varieties.
----●----● Emerged to adult stage, ----○----× Nymphal stages not completed.

Table 3. The Proportion of Different Wing Forms of Adults Emerged when the Nymphs of the Three Biotypes Were Reared on Seedlings of Susceptible and Resistant Rice Varieties

Variety	Biotype	% Adults emerged			
		B-F[a]	B-M	M-M	Total
TN 1	1	50.0	0.0	38.9	88.9
	2	50.0	0.0	50.0	100.0
	3	45.0	0.0	45.0	90.0
IR 26	1	5.0	0.0	10.0	15.0
	2	10.5	0.0	21.1	31.6
	3	35.0	5.0	45.0	85.0
IR 40	1	11.8	5.9	29.4	47.1
	2	52.6	0.0	15.8	68.4
	3	35.0	10.0	45.0	90.0

[a] B-F = Brachypterous female; B-M = Brachypterous male; M-M = Macropterous male.

Table 4. Duration (days) of Each Nymphal Instar of the Three Biotypes Reared on Susceptible and Resistant Rice Varieties

Variety	Biotype	Nymphal instar[a]				
		I	II	III	IV	V
TN 1	1	3.0 a	2.1 a	2.0 b	2.5 a	3.7 ab
	2	3.0 a	2.1 a	2.1 ab	2.5 a	3.4 b
	3	3.1 a	2.1 a	2.3 a	2.2 a	3.8 a
IR 26	1	3.2 a	3.1 a	3.5 a	3.7 a	7.0 a
	2	3.0 b	2.4 b	3.5 a	3.4 a	5.0 a
	3	3.0 b	2.1 c	2.8 b	2.6 b	4.6 a
IR 40	1	3.9 a	3.6 a	2.9 a	3.3 a	5.5 a
	2	3.8 a	3.6 a	2.7 a	3.2 a	4.5 a
	3	3.0 b	2.3 b	2.5 a	2.4 b	3.6 a

[a] Values followed by a common letter are not significantly different at the 5% level according to DMRT.

Table 5. Body Weight (mg) of Newly Emerged Female Adults of Each Biotype Reared on Susceptible and Resistant Rice Varieties[a]

Biotype	TN 1	IR 26	IR 40
1	2.53 a	1.59 a	1.29 a
2	2.61 a	1.29 ab	1.97 b
3	2.54 a	1.71 b	2.22 c

[a] Values followed by the same letter are not significantly different at the 5% level according to DMRT.

development of biotypes 1 and 3 on IR 40 were highly significant. These results are summarized in Tables 3 to 5, and Fig. 2.

Reproduction

There were significant differences in the number of progeny produced on each susceptible and resistant variety of the biotypes (Table 6). On IR 24, biotypes 1 and 2 exhibited equally high reproductivity, whereas that of biotype 3 was apparently lower than those of the other biotypes. Eighty per cent of biotypes 1 and 2 produced about 350–450 progeny over 25 days. In biotype 3 only half of the pairs tested were fertile and produced about 300 progeny on the average. It was, therefore, estimated that the reproductive potential of the biotype 3 population on susceptible varieties was about half the level of that of biotypes 1 and 2. On IR 26 and IR 40, only biotypes 2 and 3 reproduced as many progeny as those produced on IR 24, respectively. Although a few pairs of those biotypes could reproduce exceptionally well on these resistant varieties, the reproductivities of biotypes 1 and 3 on IR 26, and those of biotypes 1 and 2 on IR 40 were almost negligible (Table 6).

Reciprocal mating between biotypes 1 and 3 indicated that the low fecundity

Table 6. Number of Progeny Produced by the Three Biotypes on Susceptible and Resistant Rice Varieties

	IR 24			IR 26			IR 40		
	Biotype 1	2	3	1	2	3	1	2	3
Total no. pairs tested	20	20	20	20	20	20	20	20	20
No. fertile pairs	17	16	9	3	19	4	6	6	16
Av. no. progeny produced by fertile pairs[a]	367	442	309	310	348	191	72	144	228
Av. no. progeny produced by all the pairs tested[b]	309 b	354 b	139 a	46 a	330 b	38 a	22 a	43 a	182 b

[a] There was no significant difference among biotypes on each variety at the 5% level according to Q test.

[b] Data transformed to $\sqrt{X+1}$ for analysis but actual mean values are presented here. Values in each variety followed by the same letter are not significantly different at the 1% level according to DMRT.

Table 7. Population Buildup of the Three Biotypes on IR 24 Plants

Biotype	1	2	3
Total no. hills (A)	81	81	81
No. hills hopperburned (B)	21	36	0
Av. no. insects sampled from healthy hills (C)	283	503	73
Estimated population density per hill[a]	210	279	73

[a] C(A-B)/A

of biotype 3 was largely attributed to the females. Biotype 1 females mating with biotype 3 males produced 256 progeny on the average, while the biotype 3 females mating with biotype 1 males produced only 130 progeny.

The lower reproductive potential of biotype 3 was further revealed by small paddy plot experiments. When 50 pairs of adults of the three biotypes were released in separate paddy plot in which early tillering stage IR 24 plants were growing, hopperburn symptoms appeared in the middle of the biotype 1 and 2 plots after 2 months. The average numbers of insects per hill were 210 and 279 in the plots with biotypes 1 and 2, respectively, at that time (Table 7). On the contrary, no visible damage was observed in the biotype 3 plot, where the insect density was 73 per hill.

DISCUSSION

It has been shown that the resistance to the brown planthopper in rice varieties is mainly governed by chemicals in the phloem tissues that inhibit insect sucking (SŌGAWA and PATHAK, 1970). It was further corroborated by the fact that the amount of honeydew excreted and the degree of resistance in rice varieties were negatively correlated (KARIM, 1975). Non-preference and antibiotic phenomena observed on resistant varieties were also considered to be primarily caused by the gustatory blockage of sucking (SŌGAWA and PATHAK, 1970).

The present experiments revealed a positive correlation between the amounts of honeydew excreted and the ability to infest particular resistant varieties by each biotype population. This suggest that honeydew excretion is a useful criterion for distinguishing biotype populations. Several methods of quantifying the honeydew excretion by the brown planthopper were reported by PAGUIA et al. (1980). However, a wide range of individual variation in daily honeydew excretion was present within each biotype population, and the individual variations overlapped considerably among the biotypes. This made it difficult to differentiate biotypes on the basis of honeydew measurement on an individual basis.

Biotypic variations were also manifested by differential reproductive potentials on resistant varieties. Such differing performances of the biotypes on resistant varieties seemed to be mainly due to their differential ability to feed on resistant varieties. It was, however, noticed that a small proportion of individuals were retained in each biotype population, which could reproduce well on varieties resistant to those biotypes. At the same time, it was also suggested that the biotype 3 population carried a reproductive disadvantage, as has been pointed out at IRRI (1977).

The present result that biotype 2 preferred IR 24 to IR 26 despite its improved ability to feed and reproduce on IR 26 may indicate that this biotype is not so highly adapted to IR 26 as biotype 3 is to IR 40. However, the poor development of biotype 2 nymphs on the IR 26 seedlings may be due to an unkown effect of that variety at the seedling stage, because IMAN (1978) reported that biotype 2 nymphs can develop on Mudgo seedlings aged 15 days as well as on TN 1 seedlings.

From these results it can be concluded that the populations of the three biotypes were clearly distinguished from one another on the basis of their averaged abilities to feed and reproduce on the different rice varieties, in spite of the existence of a wide range of individual variations in physiological traits within each biotype population.

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