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## *THE BROWN PLANTHOPPER, NILAPARVATA LUGENS (STÅL) (HOMOPTERA, DELPHACIDAE) AT THE IRRI, THE PHILIPPINES*

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# STUDIES ON THE HOST RESISTANCE-BREAKING BIOTYPES OF THE BROWN PLANTHOPPER, *NILAPARVATA LUGENS* (STÅL) (HOMOPTERA, DELPHACIDAE) AT THE IRRI, THE PHILIPPINES

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1982

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# INTRODUCTION

The brown planthopper, *Nilaparvata lugens* (Stål) (hereafter called BPH), has become a serious threat to rice production throughout tropical and sub-tropical Asia with the spread of high yielding rice varieties and intensive cultural practices since about 1970 (Dyck and Thomas, 1979). The BPH causes severe sucking damage to rice crops not only by intensive sap sucking (Sōgawa and Cheng, 1979), but also by transmitting virus diseases (Rivera et al., 1966 ; Ling et al, 1978).

Since the first discovery of the BPH-resistant germplasm in rice in 1967 at the International Rice Research Institute (IRRI) (Pathak et al, 1969), four genes for resistance to the BPH, which are designated as *Bph 1, bph 2, Bph 3* and *bph 4,* have been identified (Athwal et al, 1971 ; Lakshminarayana and Khush, 1977). The successful incorporation of the BPH resistance into improved rice varieties offered a ray of hope to solve this insect problem. However, a few years after IR 26, the first commercial variety with *Bph 1* gene, was introduced in 1973 in areas where the BPH was of great economic importance, a BPH population capable to infest IR 26 became abundant (Varca and Feuer, 1976). This BPH population was described as biotype 2 to distinguish it from the original population which was called biotype 1 (IRRI, 1976). In the biotype 2 epidemic areas, the varieties with the *Bph 1* gene have been replaced by those carrying the *bph 2* gene such as IR 32 and IR 36. Those new varieties are now still effective in the fields, but the BPH biotype which can break down their resistance has already been developed in greenhouse and designated as biotype 3 (IRRI, 1976). The existence of such host resistance-breaking biotypes has further complicated the management of this rice pest by genetic manipulation in rice varieties.

It is a well-recognized problem that genetic diversity existing within an insect species can frequently result in populations capable of defeating host resistance by modifying their genetic make-up when the insect populations are subjected to a strong selection pressure in the form of intensive cultivation of resistant crop varieties. Generally, such populations, demes, or clones with different genetic or physiological abilities or inabilities to feed and colonize on different host species or varieties are commonly referred to as biotypes. The definition and history of the concept of biotypes have been reviewed by Eastop (1973). Terms such as "form", "race (biological race, physiological race, host race, host-determined race, etc.)", and "strain" have also synonymously been used for the populations of insects connected with host plant species or varieties. Van Emden et al (1969) have interpreted this terminology as follows for aphids : - A "form" is morphologically recognizable but is not necessarily genetically similar to others of the same form. "Strain" and "race" imply inherited differences in terms of morphology, bionomics, behavior or physiology. The term "strain" is appropriate to populations distinguished in the laboratory and not necessarily, like a "race", recognisable as a field population. The term "biotype" includes both "race" and "strain". In this regard, Sona and Gallun (1973) have mentioned in their report on the Hessian fly, *Mayetiola destructor,* biotypes that the terms "race" and "biotype" are interchangeable and defined as one or more Hessian flies that have specific phenotypes with respect to their ability or inability to survive on and stunt wheats having specific genes for resistance, whereas race phenotype is based on the plant's response to the insect's attack and the insect' s ability or inability to survive on the plants. Different geographical populations with distinct host plant preference are also described as "ecotype", but this term is more frequently used for the geographical populations with different voltinism or life cycle, as have been demonstrated with the rice stem borer, *Chilo suppressalis* (Fukaya, 1967), the rice stem

maggot, *Chlorops oryazae* (Iwata, 1963), and the European corn borer, *Ostinia mubilalis* (Sparks et al., 1966).

One of the typical and classical examples of host-determined biotypes is the codling moth, *Laspeyresia pomonella*. Although it is now known as a common pest of apples, walnuts, plums, pears and apriconts in most of the temperate climate areas of the world, it initially limited its attacks only to apples and pears in California where it was introduced in 1873. But since 1918 it has become a major pest of walnuts (Boyce, 1935). Phillips and Barnes (1975) demonstrated that the apple, walnut, and plum populations of the codling moth constitute well defined host-determined biotypes rather than simple normal variations within the species; and that the apple biotype is the progenitor of the walnut biotype and the walnut biotype the progenitor of the plum biotype. Another similar example is the white pine weevil, *Pissodes strobi*, which originally feeds on the white pine in the eastern nearctic regions of North America. The two western populations associated with the different host tree species have been established as a result of westward dispersion, adapting their host selection behaviors in turn to the Engelmann spruce and the Sitka spruce. These two western populations which were formerly described as *P. engelmanni* and *P. sitchensis* are now considered as geographical biotypes of *P. strobi* (Smith and Sugden, 1969; Vandersar et al, 1977). The existence of several biotypes of the grape phylloxera, *Phylloxera vitifoliae*, that cause galls on some grape varieties, necrosis on others, or both types of reaction on the same varieties have also long been known in Europe (Börner and Schilder, 1934). At least 2 biotypes of the insect have been reported in Canada (Stevenson, 1970). The most complicated genetic interactions have been demonstrated between the Hessian fly and wheat varieties (Gallun, 1977; Sona, 1978). The recent occurrence of a host resistance-breaking biotype of the chestnut gall wasp, *Dryocosmus kuriphilus*, has posed a serious problem for the chestnut cultivation in Japan (Shirura, 1972).

Many species of aphids have been found to consist of a complex of biotypes differing in their food plant preference and host resistance-breaking abilities (Eastop, 1973). The pea aphid, *Acyrthosiphon pism*, has been reported to consist of a variety of biotypic populations with distinct affinity to different leguminous plants in North America and Europe (Müller, 1962; Markkula and Roukka, 1970; Frazer, 1972; Auclair and Srivastava, 1977). In the spotted alfalfa aphid, *Therioaphis maculata*, six biotypes designated as biotypes A, B, C, E, F and H have so far been distinguished in the United States (Nielson et al., 1970; Nielson and Don, 1974). The biotypes of the spotted alfalfa aphid arose successively from the original biotype B to biotype H during the 20 years since the resistant alfalfa varieties had been grown, and the virulence in biotypes increased with the increase of selective pressure of alfalfa varieties with new resistance genes for the aphid. Two biotypes of the greenbug, *Schizaphis graminum*, designated as biotypes A and B, were separated by the reaction of certain resistant wheat varieties (Wood, 1961). In 1968, a new biotype C broke out on sorghum which can not previously been the host plant of the greenbug in the Great Plains in the United States (Harvey and Hackerott, 1969). The biotype C has been assumed to be introduced from somewhere near the Mediterranean area, where similar biotypes have been reported as a pest of sorghum (Dickson and Laird, 1969). In this regard, Müller (1966) showed that tropical populations of the greenbug are distinct biotypes. Four biotypes of the corn leaf aphid, *Rhopalosiphum maidis*, have been identified based on the differential amounts of materials taken up in resistant and susceptible sorghum varieties (Pathak and Painter, 1958). These biotypes were isolated from the White Martin sorghum, Sudan grass plant, wheat and barley in Kansas, and named KS-1, KS-2, KS-3, and KS-4, respectively. Pathak and Painter (1959)

studied the geographic distribution of the 4 biotypes in Kansas and found that they were sympatric. Two biotypes of the woolly aphid, *Eriosoma lanigerum*, have been demonstrated in South Australia, one which is able to attack varieties of apple resistant to the other (Sen Gupta and Miles, 1975). In New Zealand, a new biotype of the cabbage aphid, *Brevicoryne brassicae*, has been reported to attack formerly resistant lines of rape (Lammerink, 1968). The old biotype was designated as NZ I and the new one as NZ II. A distinct biotype of *Aphis nasturtii* has been developed in Germany, which prefers *Calla palustria* to potato that is the normal food plant of this aphid (Müller, 1969). Field collections of the rubus aphid, *Amphorophora rubi*, were classified into 4 strains by their reactions to the raspberry varieties (Briggs, 1965 ; Keep and Knight, 1967). Genetic control in the aphid was assumed to involve one dominant and one recessive gene (Briggs, 1965). In addition to the aphid biotypes mentioned above, many other examples can be cited.

The practical significance and durability of insect pest resistance in crop depend largely on the occurrence of such host resistance-breaking biotypes of insect pests. Their biological and genetical characteristics and the manner in which such biotypes develop are of considerable importance for insect pest management in agricultural ecosystems. The present studies were conducted to characterize the biological and genetic natures of the brown planthopper biotypes at the International Rice Research Institute (IRRI) from October 18, 1976 to December 25, 1979, as a part of the collaborative research project on the rice brown planthopper between the Tropical Agriculture Research Center and the IRRI.

## BROWN PLANTHOPPER BIOTYPES USED

Biotypes 1, 2 and 3 maintained as isolated inbred populations at the IRRI were used without further purification. Biotype 1 has been kept on the susceptible variety Taichung native 1 (TN 1) since 1965. Biotypes 2 and 3 have been developed from natural populations collected in Victoria, Laguna, Philippines (about 20 Km east from the IRRI) since 1974 by forced breeding on the resistant varieties Mudgo with *Bph 1* and ASE 7 with *bph 2*, respectively. The BPH populations which have been developed on Rathu Heenati with *Bph 3* and on Babawee with *bph 4* were also used in the morphological studies.

# EXPERIMENTS AND RESULTS

## I. MORPHOLOGICAL VARIATIONS AMONG BIOTYPES

### Experiments

The genital characters and dimensions of the head capsule, hind tibia, ovipositor and tegmen of 20 fresh specimens each of macropterous and brachypterous adults of both sexes sampled randomly from the three biotype populations were examined. One hundred specimens were examined to compare the frequency distribution of the lateral spine number on the hind basi-tarsus and the percentages of abnormal venation of the tegmen among the biotypes. The genitalia were taken out from the abdomens of adult specimens treated with 10% KOH for several minutes at 100°C, mounted on glass slides and then observed under a dissecting microscope.

### Results

**1. Genital characters** The genitalia, especially styles (parameres) and aedeagi in the male and lateral lobes (1st valvifers) in the female are the most important characters enabling to distinguish allied species belonging to the genus *Nilaparvata*. The apical portion of the genital styles was not bifurcated, but sharply pointed and incurvated. The inner margin of the styles was strongly excavated at the mid-portion. Aedeagus was slender, tapering apically. Its middle portion was broad. The apex of aedeagus was usually upturned. The female's lateral lobes were spatulate. Their basal portion was broader, and the inner margin showed a round shaped protrusion. These genital characters were idensical among the biotypes (Fig. 1).

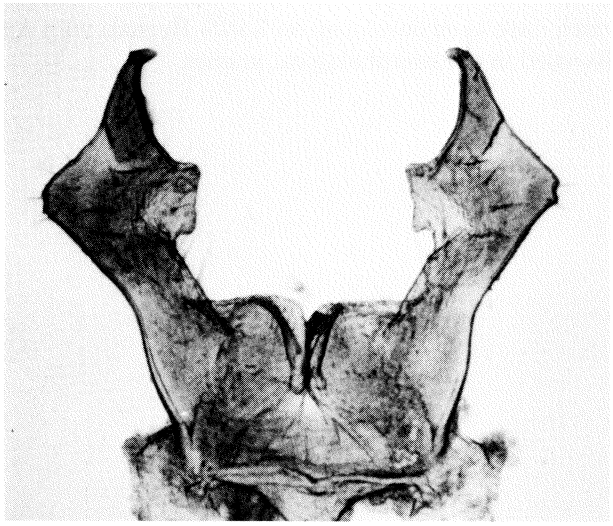

**Fig. 1. Genital style of the adult male of the BPH.**

**2. Dimensions of body portions** Dimensions of head capsule, hind tibia, ovipositor and tegmen did not differ significantly among the biotypes (Table 1). Morphometric analysis showed that the three biotypes consisted of populations, entirely overlapping.

**Table 1. Measurements of various body parts of the adults in the three BPH biotypes[a]**

| Wing form and sex | Biotype | Head width[b] (mm) | Length of hind tibia[c] (mm) | Length of ovipositor[d] (mm) | Tegmen[c] | | |
|---|---|---|---|---|---|---|---|
| | | | | | Length (mm) | Width (mm) | ratio length/ width |
| Macropterous female | 1 | 0.79 a | 1.12 b | 0.63 a | 3.54 a | 1.11 b | 3.19 a |
| | 2 | 0.79 a | 1.16 a | 0.64 a | 3.64 a | 1.18 a | 3.08 b |
| | 3 | 0.79 a | 1.13 ab | 0.60 a | 3.57 a | 1.14 b | 3.10 ab |
| Macropterous male | 1 | 0.69 a | 1.02 ab | — | 2.96 b | 0.96 b | 3.08 a |
| | 2 | 0.68 a | 1.04 a | — | 3.15 a | 1.05 a | 3.00 b |
| | 3 | 0.69 a | 1.00 b | — | 3.12 a | 1.03 a | 3.04 ab |
| Brachypterous female | 1 | 0.81 ab | 1.22 a | 0.65 a | — | — | — |
| | 2 | 0.79 b | 1.16 b | 0.66 a | — | — | — |
| | 3 | 0.82 a | 1.20 a | 0.64 a | — | — | — |
| Brachypterous male | 1 | 0.69 a | 1.06 a | — | — | — | — |
| | 2 | 0.69 a | 1.01 b | — | — | — | — |
| | 3 | 0.69 a | 1.04 a | — | — | — | — |

[a] Twenty insects were examined for each group. In each group of the three biotypes, the values followed by a common letter are not significantly different at the 5 % level (DMRT).
[b] Maximum length including the compound eyes.
[c] Either side of paired hind legs and tegmens were measured.
[d] Length of surrated part of the second valvula was measured.

**3. Number of spines on the hind basi-tarsus** Considerable variations were found among biotypes in the frequency distribution of the number of spines on the hind basi-tarsus of adults (Fig. 2). Although the spines are non-functional, they are an important taxonomic key for the genus *Nilaparvata*. The spine number varies from 0 to 6. The modes are generally 2 and 3 in the male and female, respectively. The number of spines on the right and left legs is unually equal, although a leg on one side may have one or two more spines than the other. The average spine number in biotypes 1 and 2, and in the population on Babawee did not differ significantly from that of natural populations at the IRRI, ranging from 2.76 to 2.93 in the female and 2.12 to 2.33 in the male. Their frequency patterns gave normal distribution curves. The frequncy distributions for the spine number in biotype 3 and for the population on Rathu Heenati showed skewness toward smaller numbers of spines. Skewness was greater for biotype 3. Consequently, the average spine number in biotype 3 was significantly lower on the average (2.18 to 2.39 for the female, and 1.64 to 1.83 for the male) than in biotype 1 or the natural population (Table 2, Fig. 3).

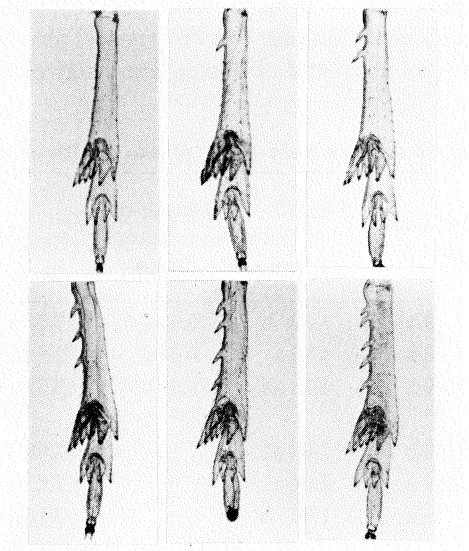

Fig. 2. Hind basi-tarsus of the BPH adults bearing different numbers of spines.

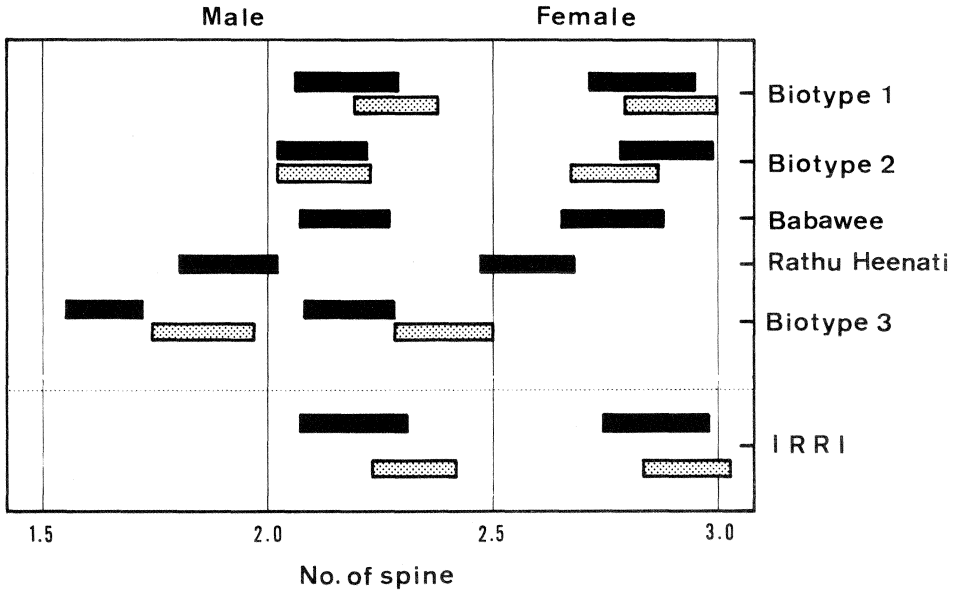


Fig. 3. Confidence rage (95%) for the average number of spines on the hind basi-tarsus of biotypes 1, 2 and 3, populations of the BPH established on Babawee and Rathu heenati, and a natural population at IRRI. ■ brachypterous form, ▨ macropterous form.

**Table 2. Average number of spines on the basal segment of hind tarsi in the three BPH biotypes[a]**

| Replication | Brachypterous form | | | | | | Macropterous form | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | | | Male | | | Female | | | Male | | |
| | Biotype | | | Biotype | | | Biotype | | | Biotype | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 1 | 2.60b | 2.98a | 2.35b | 2.05a | 2.02a | 1.70b | 3.00a | 2.90a | 2.18b | 2.15a | 2.25a | 1.78b |
| 2 | 2.93a | 2.83a | 2.00b | 2.10b | 2.45a | 1.68c | 3.18a | 2.88a | 2.35b | 2.23a | 2.05a | 1.98a |
| 3 | 3.05a | 3.05a | 2.15b | 2.35a | 1.90b | 1.73b | 2.85a | 2.73ab | 2.40b | 2.33a | 2.08a | 1.68b |
| 4 | 2.78a | 3.03a | 2.18b | 2.33a | 2.05a | 1.48b | 2.63a | 2.68a | 2.38a | 2.30a | 1.98ab | 1.78b |
| 5 | 2.80a | 2.55ab | 2.23b | 2.05a | 2.18a | 1.60b | 2.83a | 2.70a | 2.63a | 2.42a | 2.28a | 1.95b |
| Mean | 2.83a | 2.88a | 2.18b | 2.18a | 2.12a | 1.64b | 2.90a | 2.78a | 2.39b | 2.31a | 2.12b | 1.83c |

[a] In each replication for each group of the three biotypes, the values followed by a common letter are not significantly different at the 5% level (DMRT). Each replication consists of 20 pairs of hind legs.

**4. Venation in the tegmen** More than 20 types of abnormality of venation in the tegmen were observed. Among these, the loss of the M2b vein, with a lack of the 4th apical cell, was the abnormality most frequently detected (Fig. 4). It appeared more frequently in biotype 3 (13.5% in the female, 17% in the male) than in biotypes 1 and 2 (5-6% in the female, 7-10% in the male).

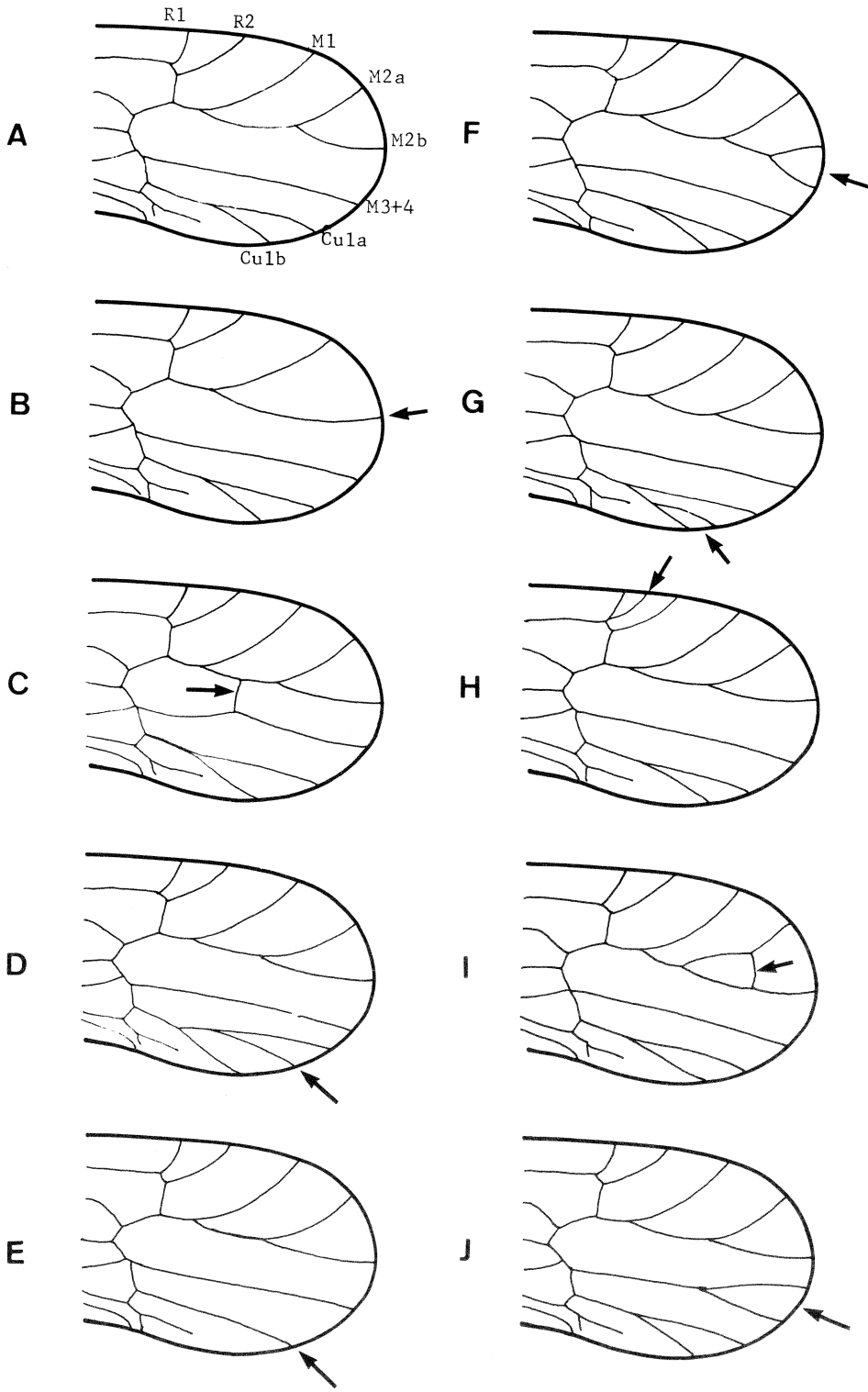


Fig. 4. Examples of abnormal venation in the tegmen of the BPH biotypes. A, normal; B, destitutus of M2b; C, extra cross vein between M2 and M3+4; D, extra branch from cu1a; E, destitutus of Cu1b; F, extra branch from M2b: G, extra branch from Cu1b; H, extra branch from R1; I, extra cross vein between M2a and M2b; J, extra branch from M3+4.

# II. ELECTROPHORETIC VARIATIONS IN ESTERASE AMONG BIOTYPES

## Experiments

About 100 macropterous and brachypterous adult males of the three biotypes and natural population at the IRRI were used. Insects were individually crushed in 10 $\mu$l of distilled water. The insect homogenate was used as enzyme solution. Agar gel electrophoresis was carried out as described by Yushima (1968). The medium for enzyme separations contained 0.7g of agar and 0.7g of polyvinylpyrrolidine in 100ml of phosphate buffer (pH 6.8, ionic strength 0. 015). The agar medium was spread over glass plates (14×10cm) as a 0.9mm thick layer. Filter paper pieces (0.5×5mm) dipped in the insect homogenates were placed on the original line of the agar gel plates for 1 hour at 5°C. After removing the filter paper pieces from the agar gel plates, electrophoresis was performed in ice-cooled hexane for about 3 hours at a constant voltage of 200V. The ionic strength of the buffer solutions in electrode vessels was adjusted at $\mu=0.05$. After electrophoresis the gels were treated with a mixture of 0.5% $\beta$-naphtyl-acetate and 0.2% naphthanildiazoblue B (1 : 5 v/v) at 37°C for 15 minutes.

## Results

Six electrophoretic phenotypes were detected and designated as types A to E (Fig. 5). The zymogram of each type was composed of three to six esterase bands with different mobility toward the anode side. All types have three fast mobile bands, E4 to E6. The enzyme activity of the bands, particularly of E4, was much stronger than that of the slow mobile E1 to E3

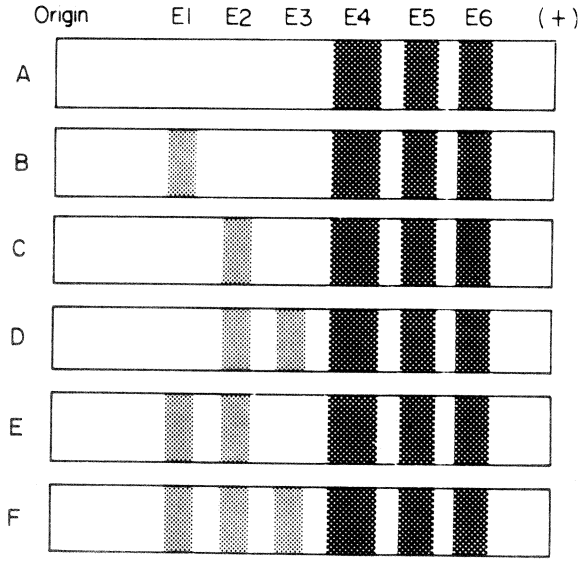


**Fig. 5. Esterase polymorphism in adult males of the BPH biotypes.**

bands. Type C was always predominant in all biotypes and in the natural population. Type E was also invariably detected in all biotypes, although much less frequently than type C. The frequencies of these two electrophoretic phenotypes in biotypes 1 and 3 were about equal; 60 to 70% for type C and 15 to 30% for type E. The percentages of type E in biotype 2 and in the natural population were apparently lower, about 10%, than those in biotypes 1 and 3. It was interesting to observe that type D with an esterase E3 band appeared almost exclusively in both brachypterous and macropterous forms of biotype 2 at a frequency of about 10% (Table 3). The occasional occurrence of type F carrying E3 band in biotype 2 may be liked with the substantial occurrence of type D. Because the enzyme activity of type A was usually weak, it is doubtful that it is a particular electrophoretic phenotype. The type B was detected rather exceptionally only in biotype 1.

**Table 3. Percentages of different electrophoretic variants in the three biotypes and a natural population of the BPH at IRRI**

| Electrophoretic phenotypes[a] | Biotype 1 | | Biotype 2 | | Biotype 3 | | Natural population |
|---|---|---|---|---|---|---|---|
| | B[b] | M | B | M | B | M | M |
| A | 9.4 | 10.2 | 12.3 | 1.9 | 8.5 | 13.0 | 1.9 |
| B | 1.9 | 0.9 | 0 | 0 | 0 | 0 | 0 |
| C | 54.8 | 68.5 | 63.2 | 79.3 | 70.8 | 63.0 | 89.8 |
| D | 0 | 0.9 | 11.3 | 9.4 | 0.9 | 0.9 | 0 |
| E | 29.2 | 16.7 | 11.3 | 7.5 | 18.9 | 23.1 | 8.3 |
| F | 0 | 0 | 1.9 | 1.9 | 0.9 | 0 | 0 |
| No activity | 4.7 | 2.8 | 0 | 0 | 0 | 0 | 0 |

[a] See Fig. 5.
[b] B, brachypterous form; M, macropterous form.

# III. BIOCHEMICAL VARIATIONS AMONG BIOTYPES

**Experiments**

**Analyses of amino acids and sugars** Fifty newly emerged brachypterous females were homogenized with 1ml of 80% ethanol in a glass homogenizer and centrifuged at 3000rpm for 10 minutes. The supernatant obtained was used for qualitative and quantitative assays for amino acids and sugars. Aliquots of 0.25ml of the supernatant were condensed by evaporating ethanol and spotted on silica gel G plates, 20×20cm for amino acid analysis, and 20×10cm for suger analysis. The plates for amino acids were developed bi-dimensionally in phenol-0.08% ammonia (4 : 1 v/v) and *n*-butanol-actic acid-water (4 : 1 : 2v/v). The plates for sugars were developed in *n*-butanol-acetic acid-water (4 : 1 : 2 v/v) by the ascending method. The separated spots of amino acids and sugars were visualized with a 0.1% ninhydrin acetone solution and 1% anilinechloride ethanol solution, respectively.

For the colorimetric assay for amino acids, the supernatant mentioned above was diluted 25 fold with citrate buffer (pH 5.0). Aliquots of 1ml of the diluted supernatant were transferred into test tubes, mixed with 1ml each of 0.5% ninhydrine and 0.2% stannous chloride citrate buffer solutions and 2ml of citrate buffer, and heated in boiling water for 20 minutes. After cooling, color intensity was measured at 570nm.

For the colorimetric assay for sugars, the supernatant was diluted 5 fold with distilled water. Aliquots of 1ml of the diluted supernatant were mixed with 1ml of 5% phenol and 5ml of concentrated sulfuric acid by agitating strongly. After cooling, color intensity was measured at 490 nm.

**Analyses of lipids and external wax** About 150 newly emerged brachypterous females were sampled from the three biotypes, dried over anhydrous $CaCl_2$ in vacuum desiccator for 2 days. The dried samples were ground finely and extracted 3 times with diethylether. The 3 extracts were pooled and concentrated at 40°C, and spotted on silica gel G plates of 20× 20cm. Petroleum ether-diethylether-acetic acid (80 : 30 : 1 v/v) was used as a solvent system to separate neutral lipids. Lipid spots were detected by heating the plates at 110°C after spraying with 50% sulfuric acid.

For polar lipid analyses, about 500 fresh brachypterous females were extracted 3 times with a chloroform-methanol mixture (2 : 1 v/v). The combined chloroform-methanol extracts were washed by shaking thoroughly with one fifth volume of distilled water. After formation of the two-phase system, the lower phase was collected, dehydrated over anhydrous sodium sulfate, and concentrated by evaporating the solvent at 50°C. The lipids extracted were first separated by mass thin layer chromatography on silica gel G with petroleum ether-diethylether-acetic acid (90 : 10 : 1 v/v). The polar lipids remaining near the original line on the silica gel G plates were scraped off and extracted with a chloroform-methanol mixture (2 : 1 v/v). The polar lipid extracts were then spotted again on silica gel G plates and separated with chloroform-methanol-water (65 : 25 : 4 v/v). Separated polar lipids were detected and identified by color reactions with phosphomolybdate reagent for phospholipids, ninhydrin reagent for amines, Dragendorff's reagent for cholines, and anthrone-sulfuric acid reagent for glycolipids.

For the analysis of external body wax, about 500 fresh brachypterous females were lightly washed with 20ml of *n*-hexane for 5 minutes. The hexane washings were dehydrated over anhydrous sodium sulfate and concentrated. The concentrated hexane washings were directly analysed by gas chromatography with a column packed with 2% OV-101 on chromosorb W

HR (100-120 mesh) with temperature programmed from 175°C to 300°C at 80°C per minute.

**Infrared spectrophotometry** Newly emerged brachypterous females were dried over anhydrous $CaCl_2$, and ground finely. About 1mg of the ground insect tissues was mixed with about 500mg of KBr and pressed into a pellet. The infrared absorption spectra of the pellets were measured with a diffraction grating infrared spectrophotometer DS-701G, Japan Spectroscopic Co. LTD.

**Analysis of honeydew** Ten brachypterous females of each biotype were confined on a basal portion of TN 1 plant at the early tillering stage with a plastic cage as described by Sōgawa (1970), for 24 hours at 27°C (Fig. 6A). Fifty insects for each biotype were employed for honeydew collection. Honeydew excreted on a parafilm at the bottom of the cage was collected with a capillary pipette.

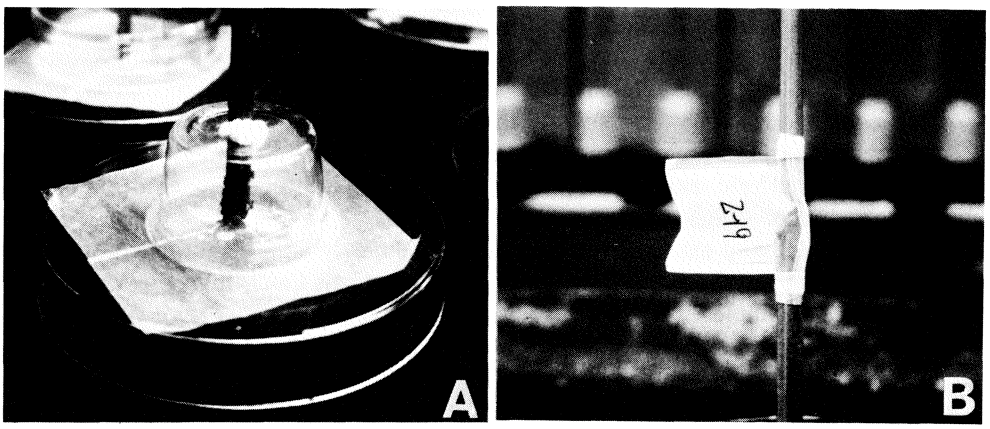


**Fig. 6. Two types of apparatus for collecting honeydews in the BPH biotypes. A, plastic cage ; B, parafilm envelope.**

For qualitative analyses of amino acid and sugar constituents in honeydew, the fresh honeydews were directly spotted on silica gel G plates, 20×20cm, and Whatman No. 1 filter paper. The silica gel G plates were developed bi-dimensionally in phenol-0.08% ammonia (4 : 1 v/v) and *n*-butanol-acetic acid-water (4 : 1 : 2 v/v). For the separation of sugars, multiple developing technique (3 times) utilizing the same solvent system, *n*-butanol-acetic acid-water (4 : 1 : 2 v/v), was applied. Amino acids and sugars were detected with ninhydrin and anilinechloride reagents, respectively.

Concentrations of amino acids and sugars in honeydews were measured by means of colorimetric assays with ninhydrin-stannous chloride and phenol-sulfuric acid reagenst, respectively, as described above in this section.

## Results

**1. Amino acids and sugars** The extracts of the fresh females contained alanine, proline and glutamic acid as major free amino acids regardless of the biotypes (Fig. 7). There were no qualitative and quantitative differences among the three biotypes (Table 4).

Trehalose and glucose were the main sugars detected in the female adults of the three biotypes (Fig. 8). The total sugar contents in the biotypes 2 and 3 tended to be lower than in the biotype 1 (Table 4).

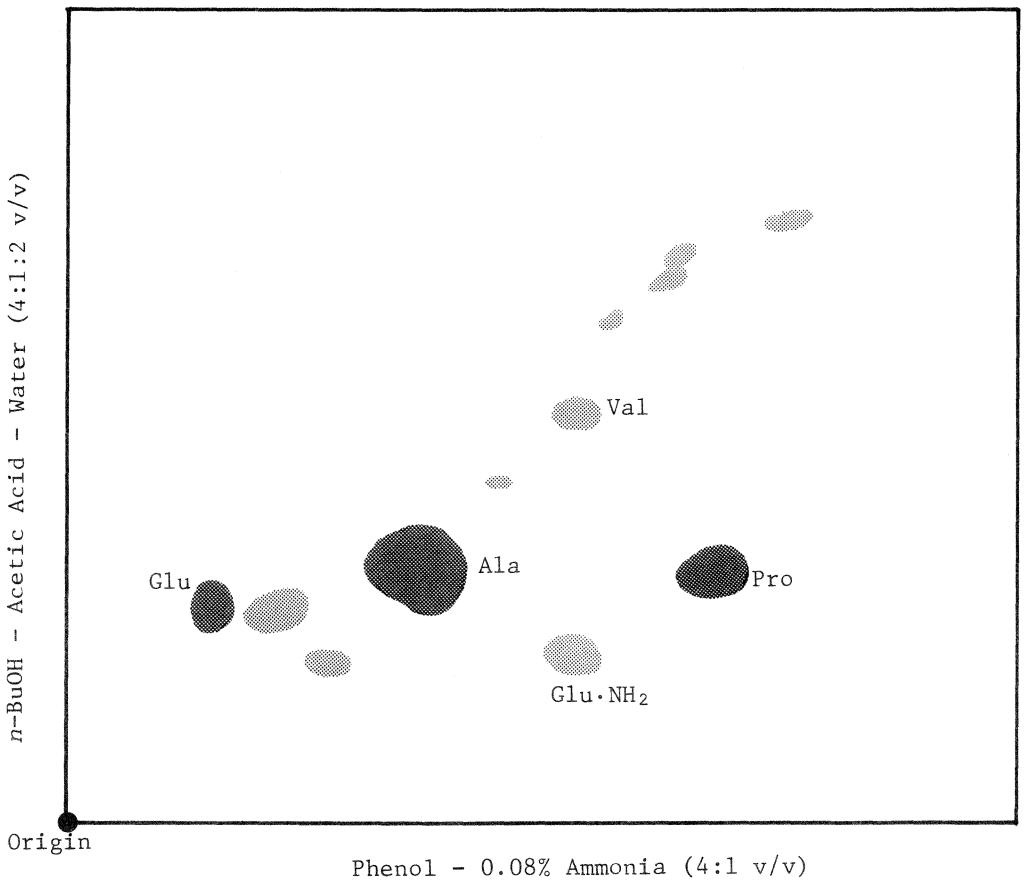


**Fig. 7. Thin layer chromatogram of amino acids in the abult females of BPH biotype l. Ala, alanine; Glu, gluta mic acid; Glu·$NH_2$, glutamine; Pro, proline; Val, valine.**

**Table 4. Relative concentration of amino acids and sugars in newly emerged females of the three BPH biotypes**

| Biotype | Relative concentration, ratio | |
|---|---|---|
| | Amino acid[a] | Sugar[b] |
| 1 | 1.00 | 1.00 |
| 2 | 1.02 | 0.75 |
| 3 | 0.96 | 0.96 |

[a] Average of 2 replications.
[b] Average of 4 replications.

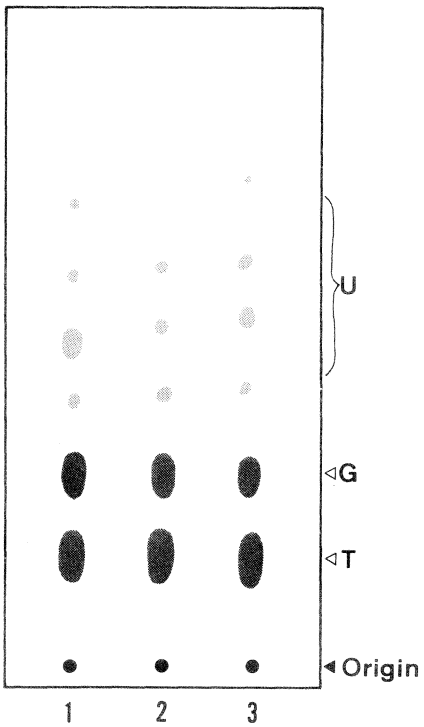


**Fig. 8. Thin layer chromatogram of sugars in the adult females of BPH biotypes 1, 2 and 3. G, glucose; T, trehalose; U, unknown substances giving yellowish and brownish spots.**

**2. Lipids and external wax** Triglycerides were found to be a major constituent in neutral lipids (Fig. 9). Phosphatidylethanolamine, phosphatidylcholine, lysophosphatidylethanolamine, sphingomyeline and lysophosphatidylcholine were identified as main polar lipids based on their color reactions against the four spraying reagents (Fig. 10, Table 5). Of these, phosphatidylcholine was the most prominent. No qualitative difference was detected in both neutral and polar lipids among the three biotypes.

Seven to ten major peaks were detected when the external wax samples from females of

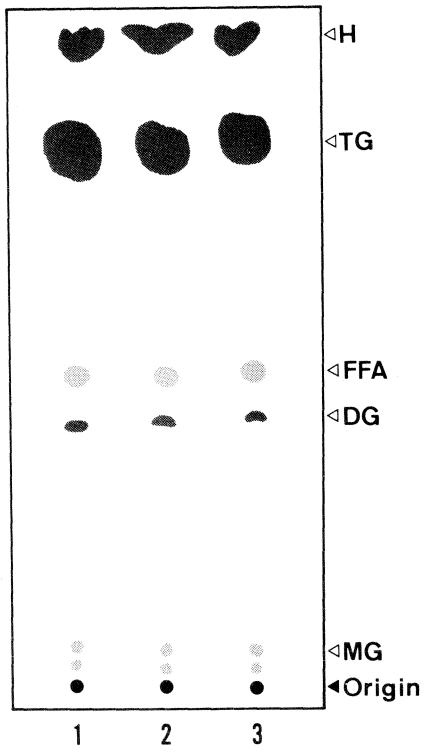


Fig. 9. **Thin layer chromatogram of neutral lipids in the adult females of BPH biotypes 1, 2 and 3. DG, diglyceride; FFA, free fatty acid; H, hydrocarbon; MG, monoglyceride; TG, triglyceride.**

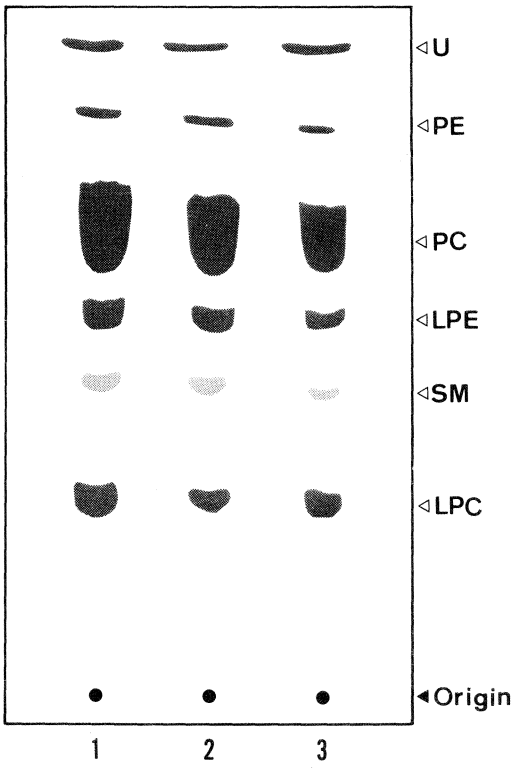


Fig. 10. **Thin layer chromatogram of polar lipids in the adult females of BPH biotypes 1, 2 and 3. The spots were detected by phosphomolybdate reagent. LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; SM, sphyingomyeline; U, unknown.**

**Table 5. Color reaction of polar lipids of females of the BHP[a]**

| Spot[b] | Phosphomolybdate reagent | Ninhydrin reagent | Dragendorff reagent | Anthrone reagent |
|---|---|---|---|---|
| LPC | +++ | − | + | − |
| SPM | ± | − | ± | − |
| LPE | + | ++ | ± | − |
| PC | +++ | − | ++ | − |
| PE | ++ | +++ | + | − |
| U | + | − | + | − |

[a] +, Positive (number of marks indicates the relative intensity of color reaction); ±, Trace; −, Negative.
[b] Refer to Fig. 10.

the three biotypes were chromatographed (Fig. 11). Qualitatively, there was no difference in the wax composition among the biotypes, except for a minor peak which appeared at about 9 minutes after the injection. The peak was apparent only in the sample from biotypes 1 and 2 (Fig. 11).

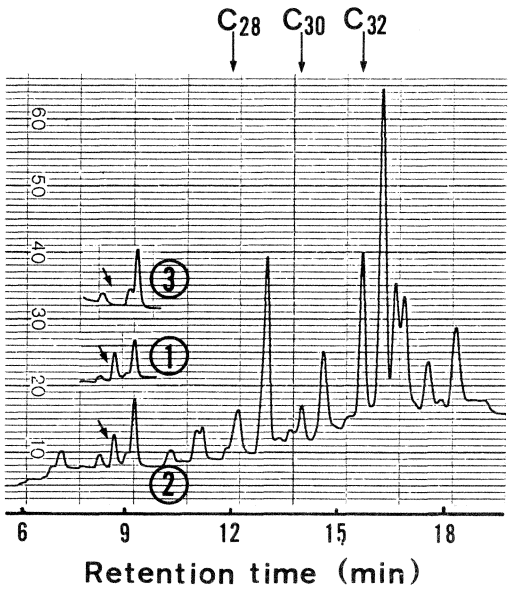


**Fig. 11. Gas chromatogram of external waxes of the adult females of BPH biotype 2. A small peak pointed by an arrow is present in biotypes 1 and 2, but not in biotype 3, Three arrows at the top indicate the peak positions of hydrocarbons with 28, 30 and 32 carbon atoms.**

**3. Infrared absorption spectra** There were three broad absorption bands at 3, 6, and 6.5nm, and a sharp absorption band at 3.4nm (Fig. 12). Samples of the three biotypes showed qualitatively identical spectra.

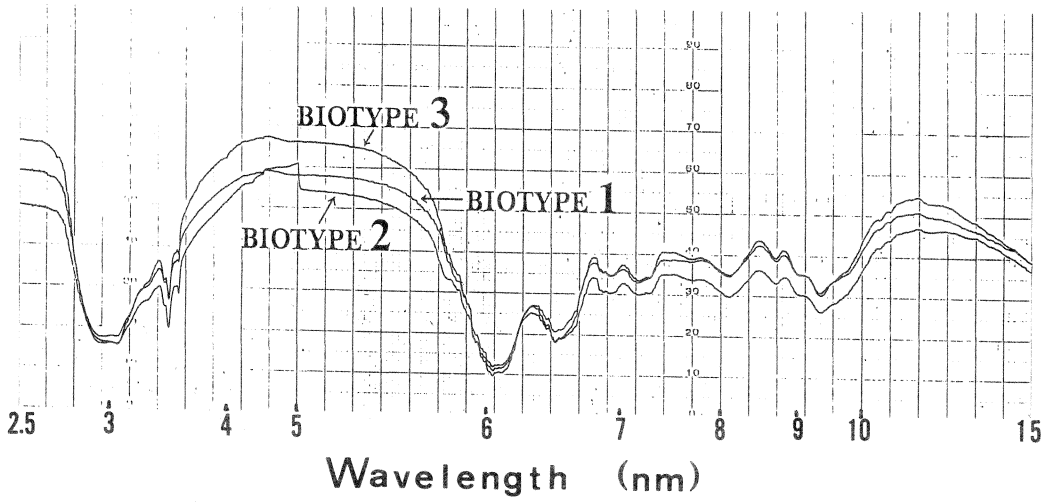


**Fig. 12. Infrared absorption spectra of the dried powder of females of the three BPH biotypes.**

**4. Honeydew** An adult female of each biotype excreted equally about 15 $\mu$l of honeydew per day containing about 9% of dry matter including 6% of sugars (as sucrose) and 0.1% of amino acids (as leucine) on young TN 1 plants (Table 6). Aspartic acid, asparagine and glutamic acid were the main amino acids in honeydews ; and sucrose, glucose and a few oligosaccharides were also detected in honeydews excreted by the three biotypes (Figs. 13, 14). No qualitative or quantitative difference was revealed among honeydews excreted by the females of the three biotypes.

**Table 6. Honeydews excreted by the females of the three BPH biotypes on TN 1 at the early tillering stage**[a]

| Biotype | Amount of honeydew excreted | Percentage of solutes | | |
|---|---|---|---|---|
| | | Total dry matter | Sugars as sucrose | Amino acids as leucine |
| | $\mu$l/insect/day | | | |
| 1 | 13.3 | 9.9 | 7.6 | 0.11 |
| 2 | 16.3 | 7.6 | 5.8 | 0.10 |
| 3 | 14.9 | 8.6 | 6.0 | 0.07 |

[a] Average of 5 replications. Each replication consisted of 10 insects.

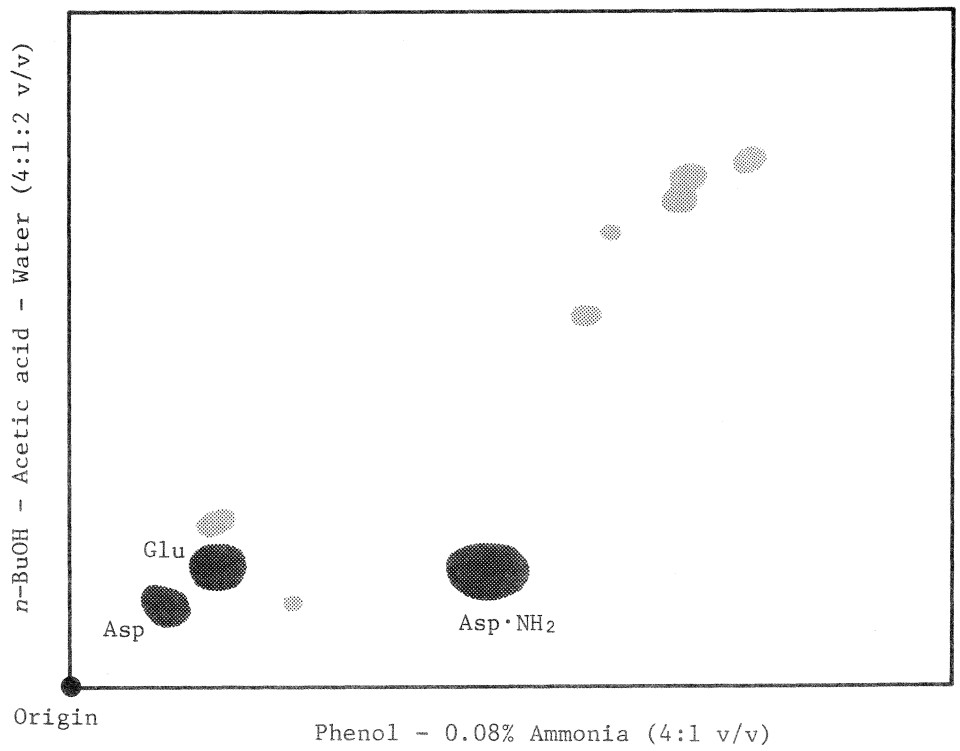


**Fig. 13. Thin layer chromatogram of amino acids in honeydew excreted by the adult females BPH biotype 2. Asp, aspartic acid ; Asp · $NH_2$, asparagine ; Glu, glutamic acid.**

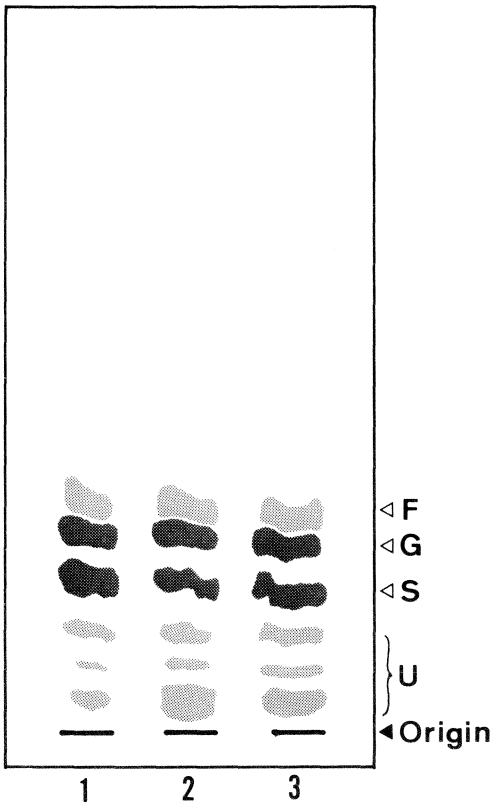


Fig. 14. Paper chromatogram of sugars in honeydew excreted by the adult females of the BPH biotypes 1, 2 and 3. F, fructse; G, glucose; S, sucrose; U, unknown oligosaccharides.

# IV. PHYSIOLOGICAL VARIATIONS AMONG BIOTYPES

## Experiments

**Tolerance to starvation** Ten newly emerged brachypterous females were confined together in a test tube (1.5×15cm) containing 1-2ml of distilled water and a piece of filter paper. The test tubes were closed with nylon gauze and kept at 27°C. The mortality of the insects was recorded periodically until all the insects died. A total of one hundred insects was tested for each biotype.

**Oxygen consumption** Oxygen consumption by the newly emerged brachypterous females of the three biotypes was measured at 30°C with a respirometer of constant pressure type (Kuramochi Seisakusho). Ten insects were confined in a vessel (25ml) after wighing. To absorb $CO_2$, 0.5ml of 20% KOH was added in a ring at the center of the vessel. The rate of oxygen consumption was recorded at 1 and 2 hours after the outset of experiments.

**Density of microsymbiotes** Ten newly emerged brachypterous females were weighed and gently ground in 10 volumes of 0.8 % saline solution with a glass homogenizer. Two aliquots of the insect homogenate were sampled and placed separately in a hemacytometer. The number of yeast-like symbiotes in 5 unit square areas of the hemacytometer was counted for each homogenate sample. The total number of symbiotes in an insect was estimated by the following formula : -

$A(B+C)/D \cdot E$

where, A=Average number of symbiotes in D,
B=Total weight of insects homgenated,
C=Volume of the saline solution used to homogenate insects,
D=Volume of insect homogenate sampled in the hemacytometer,
and E=Number of insects homogenated.

**Nymphal development and adult longevity on** *Leersia hexandra* About 10 first instar nymphs or newly emerged adults of each biotype were introduced in a test tube (2.2×20cm) containing a small amount of water and a detached stem with 2 to 3 leaves of *L. hexandra*. The test tubes were closed with nylon gauze and kept at 28°C. Mortality and nymphal development were observed daily.

**Sucking response to amino acid-sucrose solutions** Ten brachypterous females were confined in the apparatus as shown in Fig. 15, and allowed to suck each test solution through a parafilm membrane for 24 hours at 28°C. Each amino acid to be tested was dissolved at 0.1 % in a basic dietary solution which contained both 20% sucrose and 0.2% aspartic acid, and adjusted to pH 6.5 with a diluted KOH solution. The relative amount of sucking was estimated by colorimetric measurements of sugars excreted with honeydews. Honeydews excreted were collected by washing the outer surface of the parafilm membrane with 5ml of distilled water. Aliquots of 1ml of honeydew washings were subjected to colorimetric assay for sugars using phenol-sulfuric acid reagent.

## Results

**1. Tolerance to starvation** Biotypes 2 and 3, particularly the former, survived longer than biotype 1 (Fig. 16). More than 50% of the biotype 1 females died within 24 hours, whereas the mortality was only 10% for the biotype 2 at the same time. The estimated $LT_{50}$ values for biotypes 1, 2 and 3 were about 18, 40 and 26 hours, respectively.

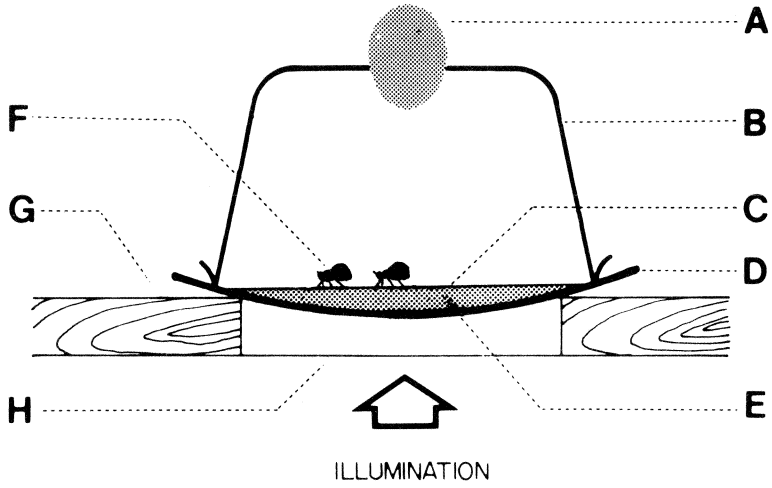


Fig. 15. Bioassay apparatus for BPH sucking on amino acid-sucrose solutions. A, sponge; B, plastic cup; C, parafilm membrane; G, watch glass; E, test solution; F, female adults; G, wooden plate at the bottom of dark cabinet; H, yellow cellophane paper.

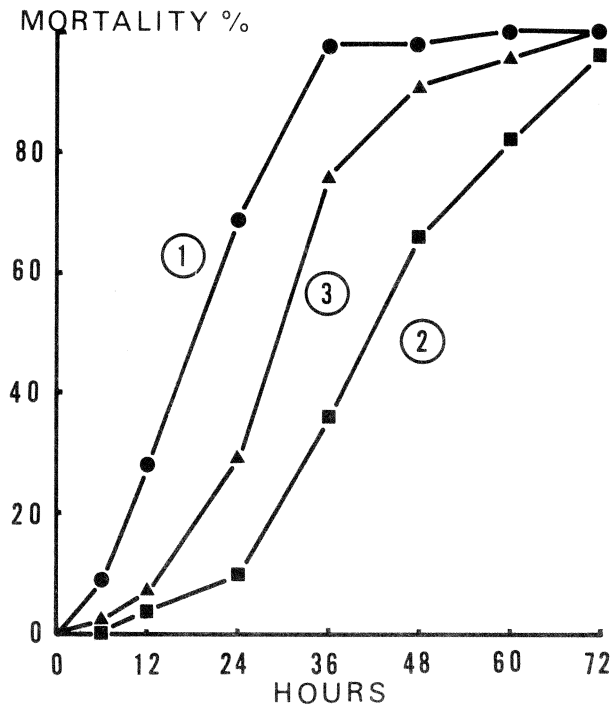


Fig. 16. Mortality trend of adult females of BPH biotypes 1, 2 and 3 under starvation.

**2. Oxygen consumption** No significant difference in the rate of oxygen consumption by the newly emerged female adults was recorded among the biotypes which were not given food (Table 7). They consumed about 0.4 to 0.5$\mu$l of $O_2$ per mg fresh weight of insects per hour at 30°C.

**Table 7. Oxygen consumption by newly emerged adult fermales of the three BPH biotypes**

| Biotype | Body weight mg/insect | $O_2$ consumption[a] $\mu$l/insect/hr at 30 °C | |
|---|---|---|---|
| | | 1 | 2 |
| 1 | 1.89±0.09 | 0.45±0.03 | 0.41±0.02 |
| 2 | 1.84±0.06 | 0.51±0.04 | 0.50±0.05 |
| 3 | 1.76±0.06 | 0.40±0.03 | 0.38±0.02 |

[a] The oxygen consumption was recoded at 1 and 2 hours after introducing the insects in the respirometer. All values are the means of 5 replications and their standard errors.

**3. Density of microsymbiotes** Yeast-like symbiotes found in the homogenate of females were elongated, ovoidal, and 10-20$\mu$ in length. The highest number of the microsymbiotes per insect about 6.7 million was recorded in biotype 1. The number of microsymbiotes in biotypes 2 and 3 was 4.6 and 5.9 million per insect, respectively. The population density per unit of fresh body weight of insects was almost indentical among the biotypes, ranging from 2.7 to 3.0 million (Table 8).

**Table 8. Population density of yeast-like symbiotes in newly emerged adult females of the three BPH biotypes**

| Biotype | No. of symbiotes/insect (No./mg), × million | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Mean±S. E. |
| 1 | 59.9 | 57.1 | 72.3 | 68.3 | 78.9 | 67.3±8.9 |
| | (2.6) | (2.6) | (3.1) | (2.9) | (3.5) | (2.9±0.4) |
| 2 | 41.8 | 44.6 | 48.6 | 39.0 | 57.2 | 46.2±7.1 |
| | (2.7) | (2.7) | (2.6) | (2.2) | (3.2) | (2.7±0.4) |
| 3 | 54.3 | 56.8 | 78.6 | 48.6 | 58.9 | 59.4±11.4 |
| | (2.5) | (2.8) | (3.2) | (2.3) | (4.0) | (3.0±0.7) |

**4. Nymphal development and adult longevity on *Leersia hexandra*** On *L. hexandra,* a possible temporary food plant, nymphs of the three biotypes experienced high mortality, ranging from 55% to 70%, particularly during the first 4 days after the transfer on *L. hexandra* and their nymphal periods were irregularly prolonged from 13 to 25 days (Table 9). No difference in nymphal growth response to this plant was found among the biotypes.

Adults of biotypes 2 and 3 tended to survive longer than biotype 1 ones on *L. hexandra* (Table 9). In particular, a significant difference in the average longevity was found between the macropterous females of biotype 1 and those of biotypes 2 and 3. The former survived for 2.5 days, while the latter 3.8 days on an average. Generally the females survived longer

than the males, and the macropterous form showed a longer life span than the brachypterous one in each biotype on *L. hexandra*.

**Table 9. Nymphal development and adult longevity of the three BPH biotypes on *Leersia hexandra***

| Biotype | Nymphal development | | Ave. adult longevity, days | | | |
|---|---|---|---|---|---|---|
| | | | Male | | Female | |
| | Mortality, % | Duration, days | B[a] | M | B | M |
| 1 | 60.0 | 15.8 | 1.4 | 1.9 | 2.2 | 2.5 |
| 2 | 70.1 | 18.4 | 1.5 | 2.6 | 2.5 | 3.8 |
| 3 | 55.0 | 17.0 | 1.6 | 2.1 | 2.5 | 3.8 |

[a] B, brachypterous from ; M, macropterous form.

**5. Sucking response to amino acid-sucrose solutions** The solutions containing asparagine, glutamine, proline and hydroxyproline were significantly more acceptable to biotypes 2 and 3 than to biotype 1 (Table 10). The sucking of biotypes 2 and 3 was also enhanced by alanine, $\gamma$-aminobutyric acid, glutamic acid, glycine and valine. All biotypes took up cysteine, leucine, methionine and serine almost equally. It is noteworthy that no amino acid-sucrose solution was more acceptable to biotype 1 than to biotypes 2 and 3.

**Table 10. Relative amount of sucking by the females of the three BPH biotypes on various amino acid-sucrose solutions[a]**

| Amino acid added to the basic solution | Biotype | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Glutamine | 1.0 | 4.0 | 3.1 |
| Asparagine | 1.0 | 3.3 | 3.7 |
| $\gamma$-Aminobutylic acid | 1.0 | 2.7 | 3.3 |
| Alanine | 1.0 | 2.2 | 3.0 |
| Proline | 1.0 | 2.7 | 2.4 |
| Glycine | 1.0 | 2.4 | 2.4 |
| Hydroxyproline | 1.0 | 1.9 | 2.1 |
| Glutamic acid | 1.0 | 1.6 | 2.3 |
| Valine | 1.0 | 1.5 | 1.7 |
| Methionine | 1.0 | 1.6 | 1.5 |
| Leucine | 1.0 | 1.4 | 1.6 |
| Serine | 1.0 | 1.1 | 1.3 |
| Cysteine | 1.0 | 1.0 | 1.0 |

[a] Average of 5 replications.

# V. INTERACTIONS BETWEEN BIOTYPES AND RICE VARIETIES

## Experiments

**Rice varieties used** Three rice varieties were utilized ; IR 24 without resistant gene, IR 26 with *Bph 1* gene, and IR 40 with *bph 2* gene. In some experiments, another susceptible variety TN 1 was used instead of IR 24. Reactions of the rice varieties tested to the three biotypes are shown in Table 11.

**Table 11. Interaction between the BPH biotypes and rice varieties used in the present experiments**

| Biotype | TN 1<br>IR 24 | Mudgo<br>IR 26 | IR 40 |
|---|---|---|---|
| 1 | S | R | R |
| 2 | S | S | R |
| 3 | S | R | S |

S, Susceptible ; R, Resistant.

**Effect of feeding on seedling growth** Pregerminated rice seeds were sown individually in test tubes (1.5×15cm) containing about 5ml of submerged soil. Two days after sowing, nowly emerged females of each biotype were introduced individually in 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, 18cm in diameter. About 20-30 females of each biotype were placed on a resistant variety at the beginning of the experiment. Then the movement of insects from the resistant variety to the susceptible one was observed periodically.

**Honeydew excretion** Twenty adult females of each biotype were confined individually on the basal portion of rice plants approximately one-month-old with an air-tight envelope made of parafilm (3×3cm), and allowed to suck on the leaf sheath for a day (Fig. 6B). 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 air-conditioned insectary at 28°C.

**Embryonic and nymphal development** Two to four hundred eggs deposited by each biotype were taken 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 period among biotypes.

To examine the nymphal development, 20 newly emerged first instar nymphs of each biotype were transferred individually to test tubes (1.5×15cm) with a root-washed one-week-old rice seedling and a small volume of tap water. The test tubes were then closed with a cotton plug, and kept in a cabinet at the 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 rice plants approximately one-month-old in cylindrical plastic cages. After 25 days, the total number of progenies 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 potential of the three biotypes on IR 24 was also compared by carrying out experiments in small paddy plots, where 3 separate paddy plots 2×2m in size covered with a net-house of 3×3×2m were used. IR 24 seedlings aged 25 days were transplanted at 20cm intervals on July 5, 1979. Fifty macropterous males and females of each biotype were released in each plot on July 25. Ten healthy hills were randomly sampled in each plot, and the number of insects on those 10 hills were counted on September 22, when the biotype 1 and 2 plots experienced partial hopperburn.

**Results**

**1. Effect of feeding on seedling growth** Both biotypes 1 and 2 caused 40-50% reduction in the seedling growth of IR 24 and IR 40. Their feeding effect was not 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 strongly than did biotypes 1 and 2 (25-50% reduction), but its feeding effect on IR 24 seedlings was smaller (30% reduction) as compared with that of biotypes 1 and 2 (40% reduction). There was no significant correlation between the ability of each biotype to infest resistant varieties and short-term feeding effect on seedling growth of the respective resistant varieties (Table 12).

**Table 12. Height (mm) of rice seedlings infested 2 days after germination by a single female of each BPH 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.

**2. Host preference** Under free-choice conditions, females of biotype 2 behaved in the same way as those of biotype 1 in avoiding to settle on IR 26. The differences in the host preference response 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. In contrast, those of biotypes 3 settled on IR 40 as shown in Fig. 17.

**3. Honeydew excretion** There was a positive correlation between the amount of honeydew excreted and the ability of each biotype to infest resistant varieties (Table 13). Females of the three biotypes excreted each as much as 40-50mg honeydew per day on TN 1 plants. On IR 26, biotype 2 females excreted nearly 30mg/day while biotype 1 and 3 females excreted less than 10mg 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 variations in daily honeydew excretion was observed within each biotype population, and the individual variatiouns overlapped among the biotypes (Fig. 18).

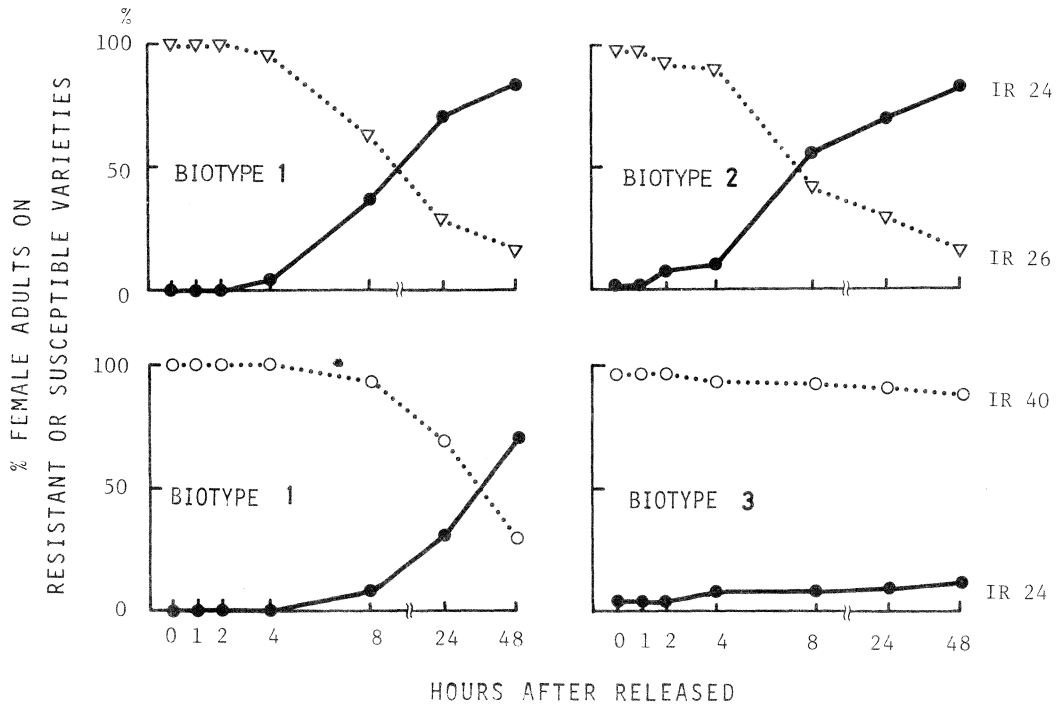


**Fig. 17. Host preference responses of biotypes 1, 2 and 3.**

**Table 13. Average weight of honeydew excreted by adult females of each BPH 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.

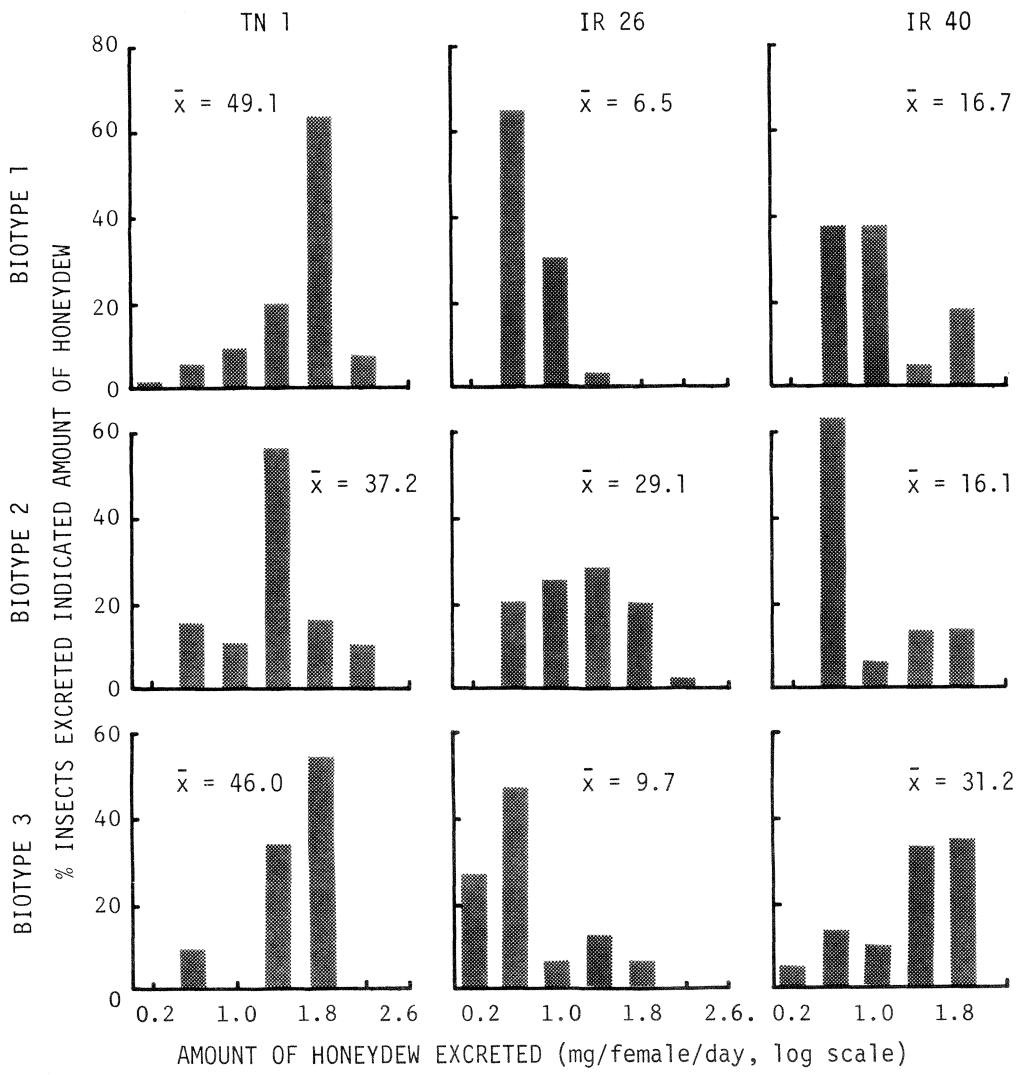


**Fig. 18. Frequency distributions of honeydew excreted by adult females of the three BPH biotypes on susceptible and resistant rice varieties.**

**4. Embryonic and nymphal development** About 33-45% eggs hatched on 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 the egg period and hatchability among the biotypes.

On the TN 1 seedlings, the 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 the nymphal duration and mortality, or in the weight of the newly emerged females. On the IR 26 and IR 40 seedlings, biotype 3 nymphs displayed the most satisfactory growth among the biotypes, although the total nymphal duration was slightly prolonged (13-

16 days), and adults emerging 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 40 varied greatly, ranging from 14 to 28 days. Such growth retardation and mortality occurred usually in the later stages of the nymphal development. Biotype 1 and 2 nymphs emerged to signficantly smaller adults on the resistant varieties. The nymphal growth response of biotypes 1 and 2 was not signficantly different on IR 26. On the other hand, differences in nymphal development of biotypes 1 and 3 on IR 40 were highly significant. These results are summarized in Tables 14 to 16, and in Fig. 19.

**Table 14. Duration (days) of each nymphal instar of the three BPH 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.

**Table 15. Relative proportion of different wing forms of adults emerged when the nymphs of the three BPH biotypes were reared on seedlings of suscptible 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 16. Body weight (mg) of newly emerged females of each BPH 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.

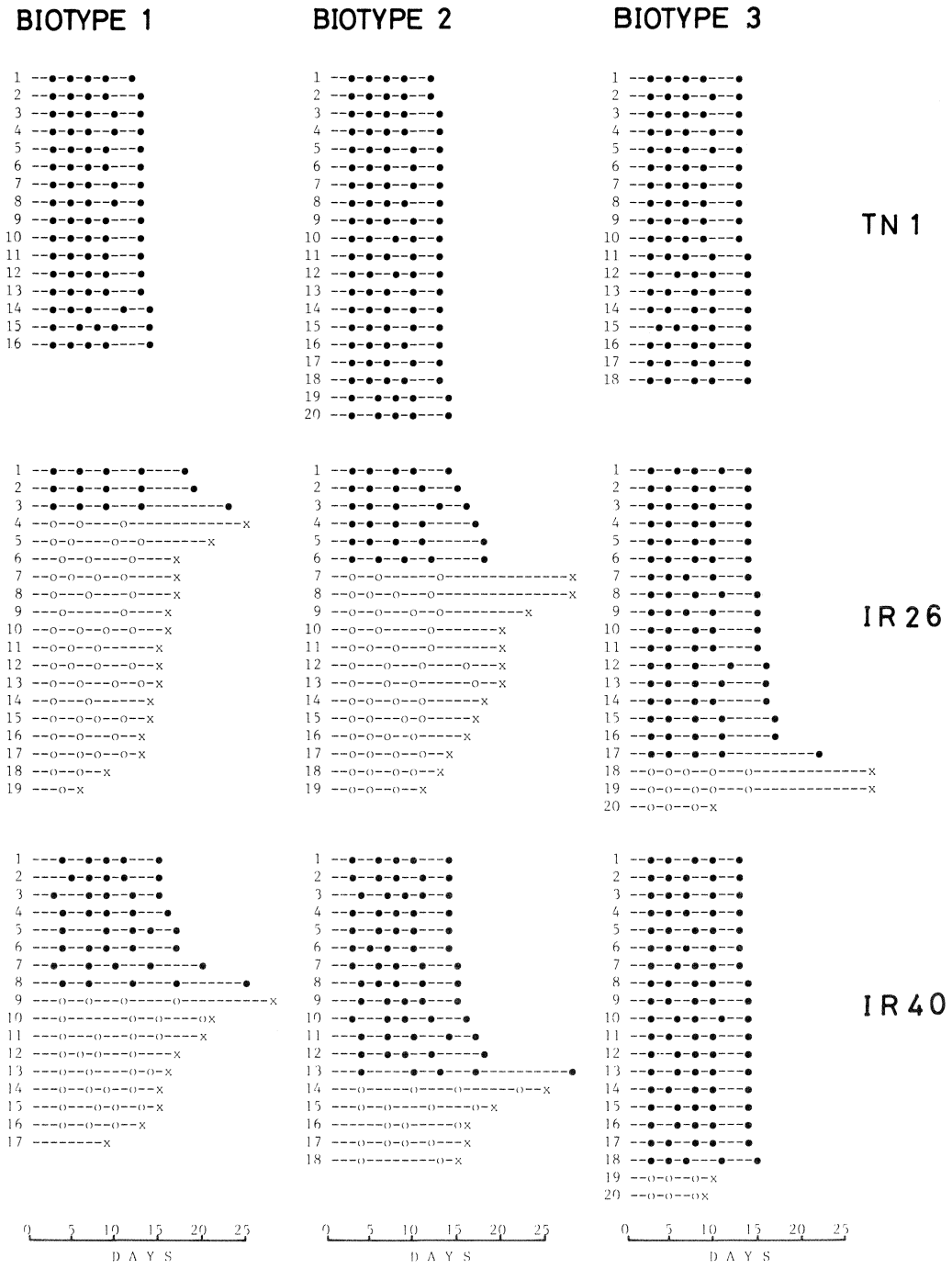


**Fig. 19. Individual records of nymphal development of BPH biotypes 1, 2 and 3 on susceptible and resistant varieties.**
**…●……● Individuals which emerged to adult stage,**
**…○……× Individuals which did not complete nymphal development and died.**
**Closed and open spots indicate the time of moulting.**

**5. Reproduction** There were significant differences in the number of progenies produced on each susceptible or resistant variety among the biotypes (Table 17). On IR 24, biotypes 1 and 2 exhibited an equally high reproductivity, whereas the reproductivity of biotype 3 was apparently lower than that of the other biotypes. Eighty per cent of the biotypes 1 and 2 produced about 350-450 progenies during 25 days. In biotype 3 only half of the pairs tested were fertile and produced about 300 progenies 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 in biotypes 1 and 2. On IR 26 and IR 40, only biotypes 2 and 3 produced as many progenies as those produced on IR 24, respectively. Although a few pairs of these biotypes could reproduce exceptionally well on the respective resistant varieties, the reproductivity of biotypes 1 and 3 on IR 26, and that of biotypes 1 and 2 on IR 40 was almost negligible (Table 17).

**Table 17. Number of progenies produced by the three BPH 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 paris | 17 | 16 | 9 | 3 | 19 | 4 | 6 | 6 | 16 |
| Av. no. progenies produced by fertile pairs [a] | 367 | 442 | 309 | 310 | 348 | 191 | 72 | 144 | 228 |
| Av. no. progenies produced by all the paris tested[b] | 309b | 354b | 139a | 46a | 330b | 38a | 22a | 43a | 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 $\sqrt{n+1}$ 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.

Reciprocal mating between biotypes 1 and 3 indicated that the low fecundity of biotype 3 was largely due to the females. The biotype 1 females mated with biotype 3 males produced 256 progenies on the average, while the biotype 3 females mated with biotype 1 male produced only 130 progenies.

The lower reproductive potential of biotype 3 was further revealed by the small paddy plot experiments. When 50 pairs of adults of each biotype were released in a separate paddy plot in which IR 24 plants at the early tillering stage were growing, hopperburn symptoms appeared in the plots with biotypes 1 and 2 after 2 months. Average numbers of insects per hill were 210 and 279 in the plots with biotypes 1 and 2, respectively, at that time (Table 18). Conversely, no visible damage was observed in the plot with biotype 3, where the insect density was 73 per hill.

**Table 18. Population buildup of the three BPH biotypes on IR 24 plants**

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

[a] C(A−B)/A.

# VI. GENETIC NATURE OF BIOTYPES

## Experiments

**Rice varieties** IR 24, IR 26 and IR 40 were mainly used. In addition, TN 1 and Mudgo were used instead of IR 24 and IR 26, respectively, in some experiments.

**Hybridization** Biotypes 1, 2 and 3 maintained as inbred populations at the IRRI were used as parents without further purification. Reciprocal matings were made between biotypes 1 and 2 and between biotypes 1 and 3. The $F_1$ progenies were backcrossed to respective host resistance-breaking biotypes. The $F_2$ progenies were obtained by crossing the two $F_1$ progenies from the reciprocal matings. The $F_1$ progenies from the reciprocal crosses between biotypes 2 and 3 were also examined. Each crossing was made in group with 5 pairs of brachypterous virgin females and males collected at random. All the progenies were reared on the susceptible IR 24 plants in a greenhouse.

**Biological traits examined** The following characteristics of hybrid progenies were compared with those of their parents : - (1) host preference response, (2) feeding ability, (3) nymphal development, and (4) fecundity. Experimental procedures were the same as those described in the previous Chapter except for those for the studies on the fecundity of hybrids from crosses between biotypes 1 and 3 and between biotypes 2 and 3. Their relative fecundity was indirectly compared with that of their parents by observing their survival and the maturation of ovarian eggs. Twenty newly emerged female adults were caged on IR 40 or IR 26 plants at the tillering stage in a greenhouse, and the number of surviving and gravid females was recorded every other day up to the 12th day after caging.

## Results

**1. Host preference response** The differences in the behavioral response to IR 40 between biotypes 1 and 3 were conspicuous. Most of the females of biotype 1 did not like to settle on IR 40, and eventually moved to IR 24 within 2 days, while biotype 3 females settled on IR 40. Females of $F_1$, $F_2$ and backcross progenies from the crosses between biotypes 1 and 3 showed a response similar to that of biotype 1(Fig. 20). Females of $F_1$ progenies from reciprocal crosses between biotypes 2 and 3 also showed a response similar to that of biotype 1, which avoided to stay either on IR 26 or IR 40. Results with the hybrids from the crosses between biotypes 1 and 2 were not satisfactory because the parental differences were not significant.

**2. Feeding response** Adult females of biotype 1 excreted only 6.5 mg of honeydew per day per insect on IR 26 on the average, while those of biotype 2 excreted 29.1 mg. The $F_1$, $F_2$ and backcross progenies from the crosses between biotypes 1 and 2 excreted as little honeydew as did biotype 1 on IR 26, ranging from 5.4 mg to 9. 3 mg (Fig. 21). Similarly, the females of $F_1$, $F_2$ and backcross progenies from the crosses between biotypes 1 and 3 excreted significantly less honeydew (6.5-11.8 mg / day / insect) on IR 40 than did biotype 3 (26.0 mg / day / insect) (Fig. 21). The amount of honeydew excreted by the $F_1$'s from reciprocal crosses between biotypes 2 and 3 on IR 26 and IR 40 was significantly lower as compared with that excreted by their respective upper parent on each resistant variety (Fig. 21).

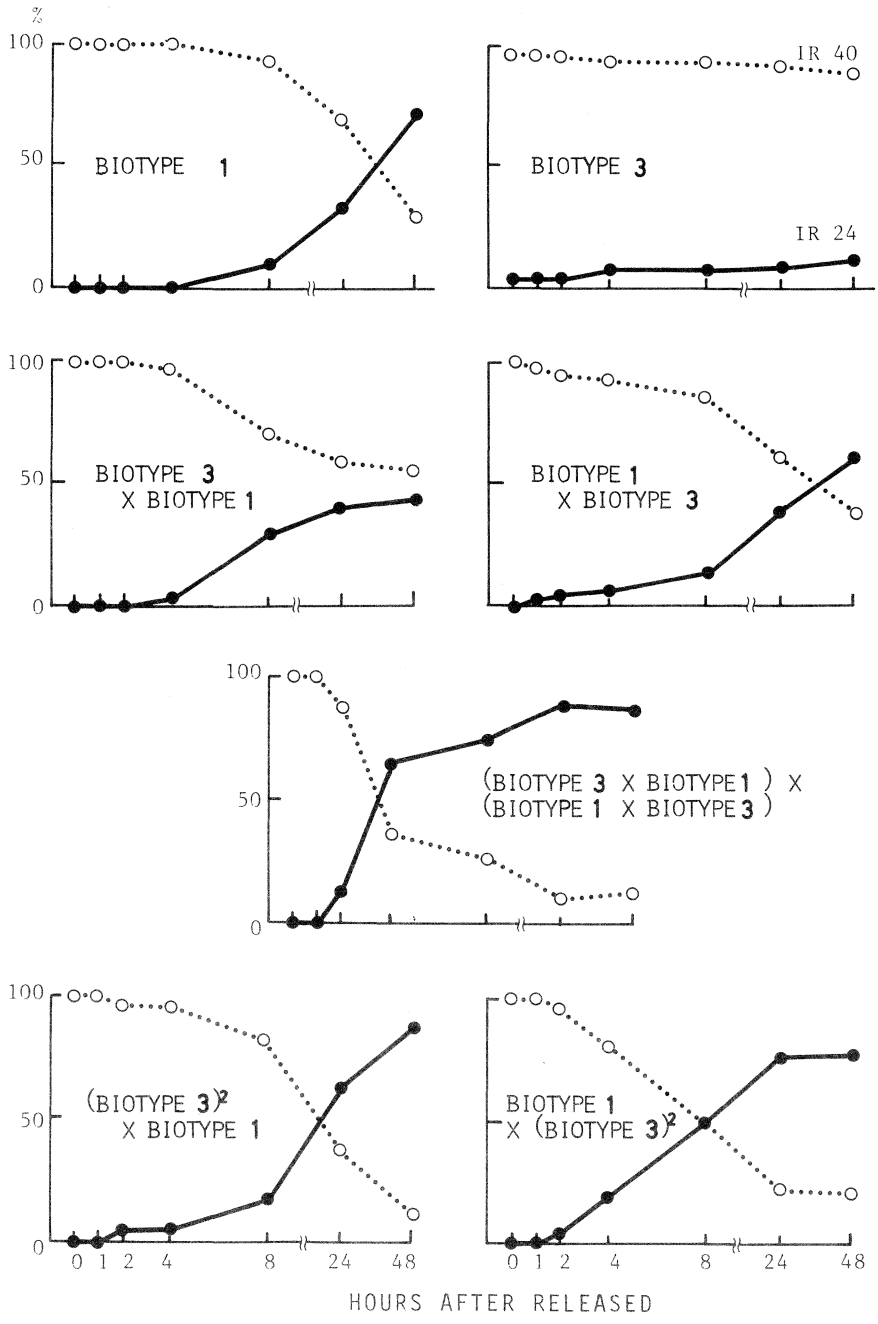


**Fig. 20. Host preference reaction of BPH biotypes 1 and 3, and their hybrid progenies.**

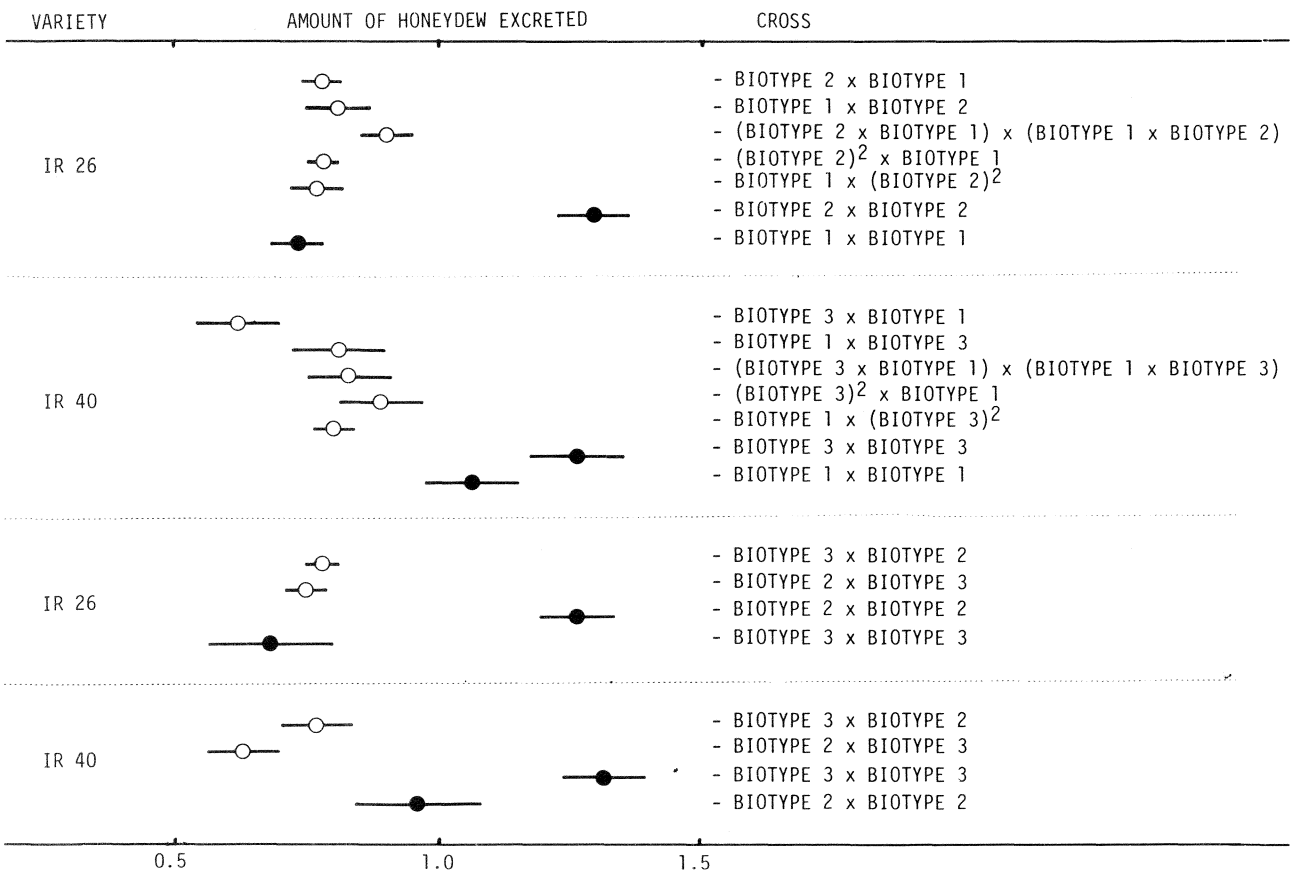


**Fig. 21. Amount of honeydew excreted by the BPH biotypes 1, 2 and 3, and their hybrid progenies on resistant varieties (mean±S. E.).**

**3. Nymphal development** No difference in the nymphal growth was observed on IR 26 seedlings among biotypes 1 and 2, and their hybrid $F_1$ progenies. Only 10-23.5% of the nymphs emerged to adults on this variety, although biotype 2 nymphs grew somehow better than biotype 1 nymphs on Mudgo seedlings. Nymphal mortality on Mudgo seedlings was 30% in biotype 2, and 68.4% in biotype 1. The nymphal mortality of two $F_1$ hybrids on Mudgo was 50 and 65%.

There were distinct differences in nymphal development on IR 40 between biotypes 1 and 3. All the nymphs of biotype 3 emerged to adults within 15 days on IR 40, while only 5% of biotype 1 nymphs reached the adult stage within the same period (Fig. 22). Another 25% of biotype 1 nymphs emerged to feeble and smaller adults taking 18-25 days to complete nymphal development. Seventy per cent of biotype 1 nymphs failed to emerge to adults. About 50-65 % of $F_1$ and $F_2$ progenies from the crosses between biotypes 1 and 3 emerged to adults on IR 40, taking 13-21 days (Fig. 22 and 23). The backcross progenies, particularly those having biotype 3 blood in the maternal side, reacted more similarly to biotype 3 as compared with $F_1$ (Fig. 22).

Generally, the two $F_1$ progenies from the cross between biotypes 2 and 3 exhibited lower nymphal mortality, but longer nymphal period on IR 26 than their parents (Table 19). On IR 40, the nymphal mortality in the two $F_1$ nymphs was as low as that in biotype 3, and their nymphal duration was similar to that of their female parent (Table 19).

**Table 19. Nymphal development of BPH biotypes 1, 2 and 3, and their hybrid progenies on resistant varieties**

| Variety | Population | No. nymphs tested | % Adults which emerged | | Nymphal duration, days | |
|---|---|---|---|---|---|---|
| | | | Total | within 14 days | Range | Average |
| IR 26 | Biotype 1×Biotype 1 | 20 | 20.0 | 0.0 | 16—26 | 23.3 |
| | Biotype 2×Biotype 2 | 20 | 10.0 | 5.0 | 13—25 | 19.0 |
| | Biotype 2×Biotype 1 | 20 | 15.0 | 0.0 | 15—29 | 23.0 |
| | Biotype 1×Biotype 2 | 17 | 23.5 | 0.0 | 17—25 | 21.3 |
| Mudgo | Biotype 1×Biotype 1 | 19 | 31.6 | 5.3 | 14—20 | 17.5 |
| | Biotype 2×Biotype 2 | 20 | 70.0 | 0.0 | 15—22 | 18.1 |
| | Biotype 2×Biotype 1 | 20 | 50.0 | 10.0 | 14—21 | 17.4 |
| | Biotype 1×Biotype 2 | 20 | 35.0 | 0.0 | 15—20 | 16.9 |
| IR 26 | Biotype 2×Biotype 2 | 20 | 33.4 | 10.0 | 14—19 | 15.7 |
| | Biotype 3×Biotype 3 | 20 | 65.0 | 20.0 | 14—18 | 15.3 |
| | Biotype 3×Biotype 2 | 20 | 65.0 | 0.0 | 15—27 | 19.8 |
| | Biotype 2×Biotype 3 | 20 | 65.0 | 0.0 | 17—27 | 19.6 |
| IR 40 | Biotype 2×Biotype 2 | 19 | 68.4 | 31.6 | 14—28 | 16.0 |
| | Biotype 3×Biotype 3 | 20 | 90.0 | 40.0 | 13—15 | 13.9 |
| | Biotype 3×Biotype 2 | 20 | 100.0 | 90.0 | 12—16 | 13.0 |
| | Biotype 2×Biotype 3 | 18 | 88.8 | 55.6 | 13—21 | 15.2 |

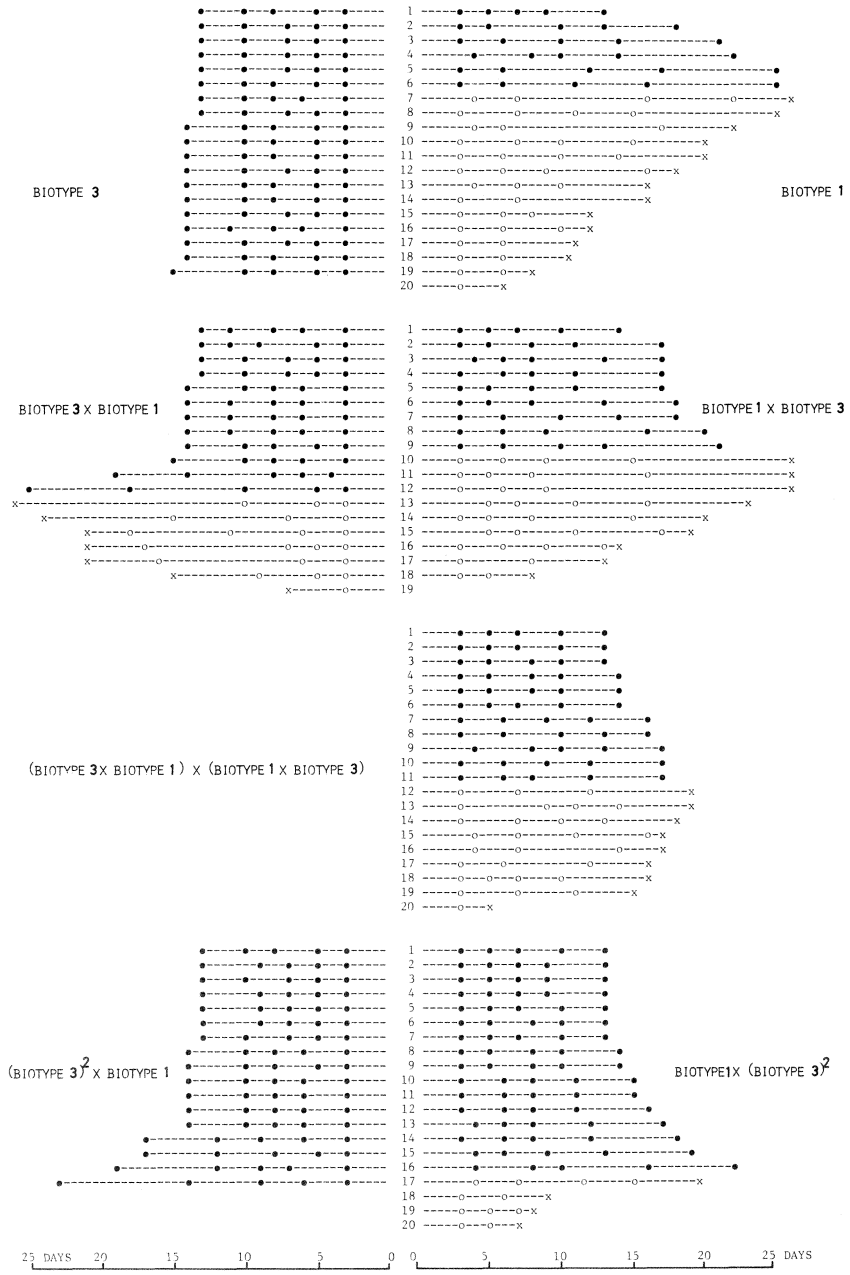


**Fig. 22. Individual records of nymphal development of biotypes 1 and 3, and their hybrid progenies on IR 40.**
**··· ● ·····, ··· ○ ····· x See Fig. 19.**

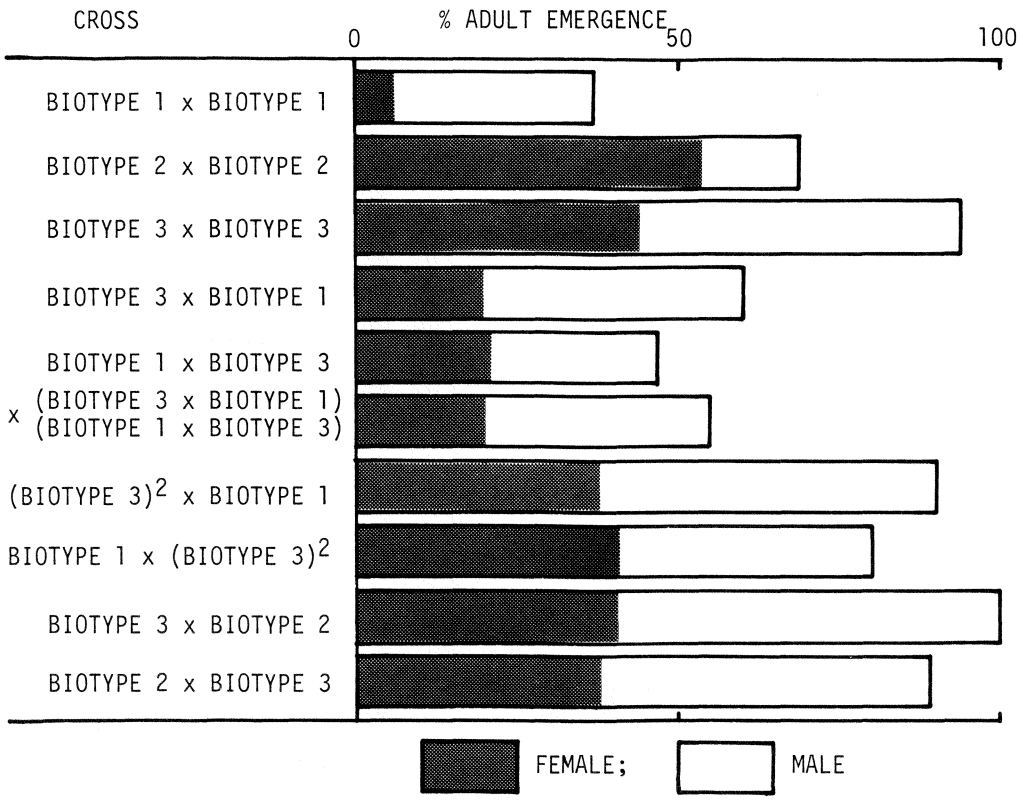


**Fig. 23. Percentages of nymphs emerged to adults in the three BPH biotypes, hybrids between biotypes 1 and 3, and those between biotypes 2 and 3 on IR 40.**

**4. Fecundity** Biotype 2 reproduced equally well on both IR 26 and IR 24, while biotype 1 failed to reproduce on the former except for a few individuals. The reproductive potential of their hybrid progenies was as low as that of biotype 1 on IR 26 (Table 20).

**Table 20. Relative fecundity of BPH biotypes 1 and 2, and their hybrid progenies on IR 26**

| Population | Pairs tested | % Pairs which reproduced | Ave. no. progenies produced by | |
|---|---|---|---|---|
| | | | reproductive pairs | all pairs |
| Biotype 2×Biotype 2 | 20 | 95 | 347.8 | 330.5 |
| Biotype 1×Biotype 1 | 20 | 15 | 309.7 | 46.5 |
| Biotype 2×Biotype 1 | 10 | 10 | 225.5 | 45.1 |
| Biotype 1×Biotype 2 | 10 | 5 | 17.0 | 1.7 |
| (Biotype 2×Biotype 1) × (Biotype 1×Biotype 2) | 10 | 10 | 66.0 | 13.2 |
| (Biotype 2)²×Biotype 1 | 10 | 5 | 45.0 | 4.5 |
| Biotype 1×(Biotype 2)² | 10 | 5 | 44.0 | 4.4 |

Remarkable variations in maturity of ovarian eggs were found among biotypes 1 and 3 and their hybrids caged on IR 40 (Fig. 24). On IR 40, newly emerged females of biotype 3 became gravid rapidly, but most of the females of biotype 1 died without becoming gravid. Only a

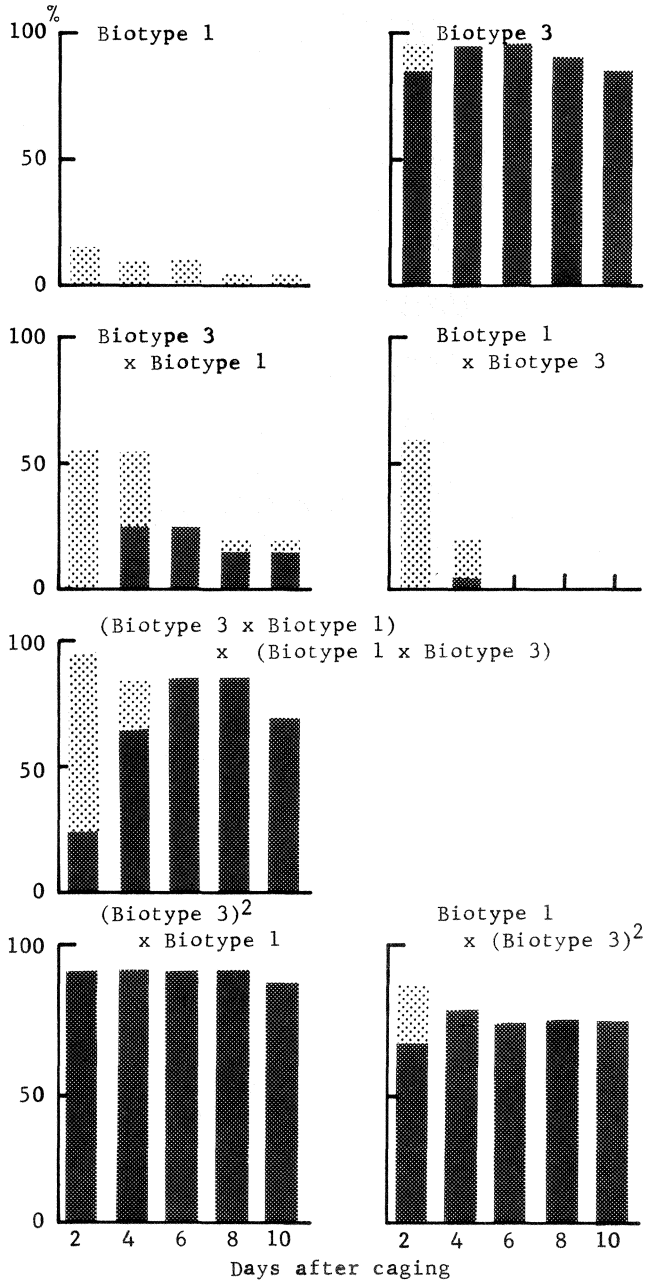


**Fig. 24. Percentage survival of females of BPH biotypes 1 and 3, and their hybrids. ▓▓Gravid, ⁘⁘Not gravid.**

few percentages of $F_1$ females from the crosses between biotypes 1 and 3 survived and became gravid, and most of them died quickly on IR 40. Both backcross progenies became gravid readily without showing a significantly high mortality on IR 40 as biotype 3 did. The survival rate of gravid $F_2$ females was unexpectedly much higher than that of $F_1$'s.

The adult females of $F_1$ progenies from the reciprocal crosses between biotypes 2 and 3 survived and became gravid at the rate of 90-100 % on IR 40, and 65-95% on IR 26 at 10 days after caging.

# DISCUSSION

## Morphological and Physiological Variations Among Biotypes

Painter (1951) stated that there appear to be two types of insect biotypes so far as resistance in plants is concerned. One is a larger and more vigorous type, whose different performance on resistant host plants is largely due to a higher rate of reproduction associated with the insect body size. The biotypes of pea aphids described by Harrington (1945) and Cartier (1959) are examples of this type. In general, their different ability to infest certain pea varieties is detected only as quantitative clonal variations which can be differentiated by statistical treatments. In the second type, certain elements of the insect physiology must fit specific elements of the plant physiology as a key fits a lock. The reactions to host varieties or species differ qualitatively among biotypes. Most of the insect biotypes belongs to this type. It was shown in the present studies that the biotypes of the BPH belong to this type.

Biotypes of the BPH as well as those of other species are generally thought to be identical in their morphological characters. This assumption was confirmed by the fact that there were no significant differences among biotypes in the dimensions of body parts nor in the genital characters. However significant variations have been found among the biotypes in the frequency distribution of the number of spines on the hind basi-tarsus of adults. This may be responsible for different modes or intensities of selection pressure operating during the development of each biotype, or for the genetic drift associated with high mortality during the initial stage of biotype development. Liquido (1978) mentioned that the computed high degree of misclassification and the extreme overlap of clusters suggests strongly that the BPH biotypes can not be discriminated morphometrically. However, about 50% and 60% of biotype descrimination in males and females, respectively, was found using the spine number of the hind basi-tarsus, the number of teeth of the tibial spur, and the genital characters (length of phallus and paramere, and number of spines in phallus for males ; length of the 1 st, 2nd and 3rd valvulae for females) in multiple discriminant analysis. Meier (1964) found that the pea aphid in Switzerland shows large variations in the number of hair on the cauda and in the percentage of those that are stunted. An attempt to apply these morphological differences to distinguish biotypes with different food-plant preferences was not successful. Morphological variations among the greenbug biotypes are distinct (Wood et al., 1969). Biotype C of the greenbug is much lighter in color. Its cornicles are yellowish-green with no blackening (one third of the distal end is black in biotypes A and B), and are not expanded apically. Winkles are present throughout the entire length of the cornicles of biotype C, while winkles were present on the basal portions only in biotypes A and B.

In addition to the conventional taxonomic methods based on the external morphology, various biochemical procedures have been employed with a view to distinguishing among allied species complex. In the present experiments, electrophoresis, infrared spectrophotometry, and paper- and thin layer chromatography did not reveal any significant or consistent biochemical differences enabling to identify the three biotypes of the BPH, except for subtle quantitative variation in esterase polymorphism. Shimura (1972) demonstrated that a host resistance-breaking biotype of the chestnut gall wasp could be clearly distinguished from the original biotype by the presence of an additional peroxidase isozyme.

Among the physiological propeties studied, the sucking response to amino acid-sucrose solutions differed significantly among the biotypes. Biotypes 2 and 3 imbibed apparently more on solutions containing amino acids which are not always major constituents in rice

plants than did biotype 1, indicating that biotypes 2 and 3 have a wider adaptability to unusual dietary substances as compared with biotype 1. No amino acid-sucrose solution was more acceptable to biotype 1 than to biotypes 2 and 3. This may partly explain the improved ability of biotypes 2 and 3 to feed on resistant varieties. Better tolerance of biotypes 2 and 3 to starvation and their slightly longer survival on *L. hexandra* may also be attributed to the same physiological property. Conversely, the invertase activity in the salivary glands of the biotype 1 has been found to be about twice as high as that of the biotypes 2 and 3 (IRRI, 1976). In addition, possible variations in the susceptibility to insecticides have been reported (Heinrichs and Valencia, 1978), whereas there is no significant difference in the ability to transmit the rice virus diseases (Aguiero and Ling, 1977a, 1977b).

**Biotype-Variety Interaction**

It has been shown that the resistance to the BPH in rice varieties is mainly governed by chemicals in the phloem tissues that inhibit insect sucking (Sōgawa and Pathak, 1970). This 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 amount of honeydew excreted and the ability to infest particular resistant varieties by each biotype population. This suggests that honeydew excretion is a useful criterion to distinguish biotype populations. Several methods to quantify the honeydew excretion by the BPH were reported by Paguia et al. (1980). However, a wide range of individual variations 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. Claridge and Hollander (1980) have also pointed out considerable variation and overlap among the BPH biotypes in weights of honeydew excreted and concluded that the nature of the biotypes is obscure.

Biotypic variations were also manifested by different reproductive potentials on resistant varieties. Different performance of the biotypes on resistant varieties seemed to be mainly due to their different ability to feed on resistant varieties. It was, however, noticed that a small proportion of individuals was retained in each biotype population, which could reproduce well on varieties resistant to the respective biotypes. At the same time, it was also suggested that the biotype 3 population carried a reproductive disadvantage, as pointed out at the IRRI (1977). In this connection it was reported that the insecticide-resistant strains of insects are frequently less viable, less fertile, and develop more slowly (see Crow, 1957). The genes conferring the ability to defeat host resistance are generally at a low frequency in the natural population before the resistant varieties are introduced in the fields. This evidence seems to indicate that individuals carrying those genes are less fit from a survival standpoint.

The present results showing 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 as highly adapted to IR 26 as biotype 3 is to IR 40. The poor development of biotype 2 nymphs on the IR 26 seedlings may be due to unknown effects of that variety at the seedling stage, because Iman (1978) reported that biotype 2 nymphs could develop on Mudgo seedlings aged 15 days as well as on TN 1 seedlings.

From the results mentioned above, it can be concluded that the populations of the three biotypes were clearly distinguished from one another on the basis of their averaged ability to

feed and reproduce on the differential rice varieties, in spite of the existence of a wide range of individual variations in these physiological traits within each biotype population.

**Genetic Nature of Biotypes**

Hybridization experiments showed that some biological characteristics of biotypes 2 and 3 were entirely lost or diluted when those biotypes were crossed with biotype 1, as shown in Table 21. All the inter-biotypic hybrids excreted as little honeydew on resistant varieties as did biotype 1. The improved feeding ability was not restored by backcrossing the hybrid $F_1$'s to their respective upper parental biotypes. Likewise, the hybrids between biotypes 1 and 3, and between biotypes 2 and 3 displayed a host preference behavior similar to that of biotype 1. These results indicate that the recessiveness of biotypes 2 and 3 against biotype 1 with respect to the ability to feed on the resistant varieties, in agreement with the previous IRRI's finding (IRRI, 1978). However, the previous results showing that biotype 3 is dominant over biotype 1 (Cheng and Chang, 1979) and biotype 2 (IRRI, 1978) were not confirmed in the present experiments.

**Table 21. Behavioral and physiological reactions of the $F_1$, $F_2$ and backcross (BC) progenies from inter-biotypic crossings on resistant varieties**

| Reaction[a] | Biotypes 1×2 | | | Biotypes 1×3 | | | Biotypes 2×3 |
|---|---|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | BC | $F_1$ | $F_2$ | BC | $F_1$ |
| Host preference | * | * | * | 1 | 1 | 1 | 1 |
| Honeydew excretion | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Nymphal development | 1 | * | * | 1—3 | 1—3 | 1—3 | 2/3 |
| Fecundity | 1 | 1 | 1 | 1 | 3>1 | 3>1 | 2/3 |

[a] 1, Reaction similar to that of biotype 1.
1—3, Reaction intermediate between that of biotypes 1 and 3.
3>1, Reaction more similar to that of biotype 3 than of biotype 1.
2/3, Reaction similar to that of biotypes 2 or 3 depending on host varieties.
*, Not tested because of no significant parental differences.

It should be pointed out that all the hybrid progenies used in the present experiments were reared on the susceptible variety IR 24, and transferred to resistant varieties at the adult stage to evaluate their immediate feeding and preference responses to them. As indicated in aphid species (e.g. Auclair, 1966; Lowe, 1973), such an abrupt change of food plants may influence the expression of the insects' genetic ability to accept low phagostimulative resistant varieties. Possible preconditioning effect by food plants during the nymphal stages may have been overlooked in the nymphal development experiments, where the first-instar nymphs were transferred to resistant varieties within a day, after hatching on the susceptible variety. In fact these experiments provided somewhat different information on the genetic nature of biotypes. The nymphal growth of hybrid $F_1$ progenies from the crosses between biotypes 1 and 3 was seemingly intermediate between that of their parents, indicating an incomplete dominance of the ability of biotype 3 nymphs to grow on resistant varieties. Also the rapid acquisition of the biotype 3 character through a single backcross suggests the involvement of relatively a few genes in this character of biotype 3.

Further experiments are needed to determine whether the indefinite segregations in the $F_2$ and backcross progenies are due to the polygenic nature of physiological traits examined or to the genetic heterogeneity of parental biotype populations. In this regard, it seems interesting to refer to the findings from the genetic studies on insect populations resistant to insecticides. According to Lichtwardt (1956) and Lichtwardt et al. (1955), the resistance factor remains unfixed in the populations in which the resistant genotypes may be heterozygous rather than homozygous, probably because of the competitive disadvantage of the homozygotes for the resistance factor ; and homozygous population is achieved only by rigorous inbreeding. Genetic analysis of insecticide resistance has shown the evidence of polygenic as well as monogenic inheritance (Crow, 1957). At the same time, however, it has been pointed out that there are examples a polygenic nature can be incriminated because the gradual acquisition of resistance and the absence of clear-cut monofactorial segregation ratios are in fact largely determined by a single factor (see Crow, 1957). Crow (1957) suggested that the best method for isolating a major factor is to carry out repeated backcrossings with selection.

**Development of Biotypes**

The evolution of BPH biotypes is an exceedingly complex process governed by the interactions of genetic and biological factors of the BPH populations, and the cultural conditions of the resistant rice varieties. The mode of genetic interaction between the BPH populations and the rice varieties is an essential genetic aspect. In addition, the genetic factors involve the dominance and initial frequency of the genes that confer the ability to overcome varietal resistance of rice.

Among the biological factors, the reproductive fitness of particular BPH populations (phenotypes) on particular rice varieties, the existence of refugia, and migration are the main influential factors in the evolution of biotypes. It seems reasonable to postulate that host resistance-breaking biotypes carry the genetic load of lowered fitness in the absence of varietal resistance in the host plants, because most populations are ordinarily dominated by biotypes which have no ability to defeat host resistance if there is no selection pressure by resistant varieties. The net effect of lowered fitness is manifested as a reproductive disadvantage. Such selective disadvantage retards biotype development or keeps the biotypic characters is an unfixed condition in a population. Similarly the presence of refugia also delays the increase of particular phenotypes adapted to particular host variety in a given population because a proportion of the population escapes selection pressure, which enables genetic diversity to be maintained in the population longer. When it is feasible to provide refugia in a target population with mosaic, mixed or alternative cultivation of the resistant varieties in maintaining selection pressure-free areas in the habitats of the BPH, or selection pressure-free periods in the life cycle of the BPH, the existence of refugia becomes a controllable operational factor to regulate the development of biotypes in the fields. Migration or dispersion, which is originally an important adaptive response of the BPH with wing dimorphism to the habitat instability, also retards the accumulation of certain phenotypes at a selection site by the influx and efflux of individuals with different phenotypes.

The intensity and mode of selection pressure by the cultivation of resistant varieties are operational factors under human control. There are several proposed strategies to control and stabilize the prevalence of BPH infestation by means of sequential release, mosaic and mixed cultivation of oligogenic or polygenic resistant varieties, or the cultivation of

multilines. These mechanisms operate as selective pressure with different levels and different modes of action on the BPH populations.

Among the factors mentioned above, the genetic and biological factors, except for refugia, are intrinsic characters of the BPH populations, which are beyond human control. It is only possible to control the evolution of biotypes through the manipulation of operational factors. It is of a practical significance to evaluate the effect of each mode of culturally operational factors on the genetic status of the existing BPH populations as well as to evaluate its effect on the control of the population level of BPH, in order to prevent the development of virulent biotypes.

As a result of genetic and biological interactions between the BPH and rice varieties, the genetic make-up of the BPH population will be shifted by accumulation and recombination of genes raising the fitness of the population on the existing resistant varieties. The elimination of offtypes, inbreeding among survivors, and reproductive competition among newly evolved genotypes are considered as major events involved in the process of biotype formation. The present experiments showed that the biological traits associated with the host resistance-breaking ability of biotypes 2 and 3 were generally lost or weakened when these were intercrossed with different biotypes, particularly with biotype 1. Because of the recessive nature of biotypes 2 and 3, and the absence of reproductive barrier among biotypes, the resistance-breaking biotypes will be unstable unless they are effectively isolated. Therefore, cultural practices aimed to avoid monoculture of particular resistant varieties and to retain the biotype 1 population will be effective in preventing the development and prevalence of particular virulent biotypes.

# SUMMARY

There were no consistent morphological and biochemical characters available as genetic markers to distinguish the three biotypes of the BPH (biotypes 1, 2 and 3) at the IRRI, except for the significant variations in the sucking response to various amino acid-sucrose solutions. Biotypes 2 and 3 appeared to have a wider acceptability to those artificial dietary substrates.

Biotypes were found to differ significantly in their host preference and feeding responses as well as nymphal development and fecundity on the resistant varieties. In particular, the average amount of honeydew excreted on resistant varieties differed among biotypes despite a wide range of individual variations. The different ability of biotypes to suck the resistant varieties was considered to be a crucial factor responsible for their different performance on the resistant varieties. Different reproductive potentials were also found among them not only on resistant varieties but also on susceptible ones. A reproductive disadvantage was loaded by biotype 3.

Hybridization experiments showed that physiological characters of biotypes 2 and 3 were inherited in a recessive or intermediate manner when these were crossed with biotype 1, indicating that the host resistance-breaking biotypes 2 and 3 were not stable in the conditions which allow the coexistence of biotype 1. It could not be clearly demonstrated whether the indefinite segregations in the $F_2$ and backcross progenies were due to the polygenic nature of the traits examined or to the genetic heterogeneity of the biotype populations used.

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## RESEARCH ARTICLES PUBLISHED

1. Sōgawa, K. (1978) Electrophoretic variations in esterase among biotypes of the brown planthopper. IRRN 3(5) : 8-9.
2. Sōgawa, K. (1978) Variations in gustatory response to amino acid-sucrose solutions among biotypes of the brown planthopper. IRRN 3(5) : 9.
3. Sōgawa, K. (1978) Quantitative morphological variations among biotypes of the brown planthopper. IRRN 3(6) : 9-10.
4. Sōgawa, K. (1980) Biological and genetic nature of biotype populations of the brown planthopper, *Nilaparvata lugens*. JARQ 14 : 186-190.
5. Sōgawa, K. (1981) Biotypic variation in the brown planthopper, *Nilaparvata lugens* (Homoptera : Delphacidae) at the IRRI, the Philippines. Appl. Ent. Zool. 16 : 129-137.
6. Sōgawa, K. (1981) Hybridization experiments on three biotypes of the brown planthopper, *Nilaparvata lugens* (Homoptera : Delphacidae) at the IRRI, the Philippines. Appl, Ent. Zool. 16 : 193-199.

# LITERATURE CITED


1. Aguiero, V. M. and K. C. Ling (1977a) Transmission of rice grassy stunt by three biotypes of *Nilaparvata lugens*. IRRN 2(6) : 12.
2. Aguiero, V. M. and K. C. Ling (1977b) Transmission of rice ragged stunt by biotypes of *Nilaparvata lugens*. IRRN 2(6) : 12.
3. Athwal, D. S., M. D. Pathak, E. H. Bacalongco and C. D. Pura (1971) Genetics of resistance to brown planthoppers and green leafhoppers in *Oryza sativa* L., Crop Sci. 11 : 745-750.
4. Auclair, J. L. (1966) Dissimilarities in the biology of the pea aphid, *Acyrthosiphon pisum* (Homoptera : Aphididae), on alfalfa and peas in New Mexico. Ann. Ent. Soc. Am. 59 : 780-786.
5. Auclair, J. L. and P. N. Srivastava (1977) Distinction de biotypes dans des populations de puceron du pois, *Acyrthosiphon pisum* (Harris), de l'Amérique du Nord. Can. J. Zool. 55 : 983-989.
6. Börner, C, and F. A. Schilder (1934) Beiträge zur Züchtung reblausund mehltaufester Reben. II Das Verhalten der Blattreblaus zu den Reben des Naumburger Sortimentes. Mitt. Biol. Reichsanst. Berlin-Dahlem 12 : 5-84 (Cited from Stevenson, A. B., 1970).
7. Boyce, A. M. (1935) The codling moth in Persian walnuts. J. Econ. Ent. 28 : 864-873.
8. Briggs, J. B. (1965) The distribution, abundance, and genetic relationships of four strains of the rubus aphid (*Amphorophora rubi* (Kalt.)) in relation to raspberry breeding. J. Hort. Sci. 40 : 109-117.
9. Cartier, J. J. (1959) Recognition of three biotypes of the pea aphid from Southern Quebec. J. Econ. Ent. 52 : 293-294.
10. Cheng, C. H. and W. L. Chang (1979) Studies on varietal resistance to the brown planthopper in Taiwan. In : *Brown Planthopper : threat to rice production in Asia.* IRRI, Los Banos, Philippines. pp. 251-271.
11. Claridge, M. F. and J. den Hollander (1980) The "biotype" of the rice brown planthopper, *Nilaparvata lugens*. Ent. exp. appl. 27 : 23-30.
12. Crow, J. F. (1957) Genetics of insect resistance to chemicals. Ann. Rev. Ent. 2 : 227-246.
13. Dickson, R. C. and E. F. Laird, Jr. (1969) Crop host preferences of greenbug biotype attacking sorghum. J. Econ. Ent. 62 : 1241.
14. Dyck, V. A. and Thomas, B. (1979) The brown planthopper problems. See Ref. 11, pp. 3-17.
15. Eastop, V. F. (1973) Biotypes of aphids. In : *Perspective in Aphid Biology*. Ed. by A. D. Lowe, Ent. Soc. New Zealand. pp. 40-51.
16. Frazer, B. D. (1972) Population dynamics and recognition of biotypes in the pea aphiid (Homoptera : Aphididae). Can. Entomol. 104 : 1729-1728.
17. Fukaya, M. (1967) Physiology of rice stem borers, including hibernation and diapause. In : *The Major Insect Pests of the Rice Plant*. Jocns Hopkins Press, Baltimore, U. S. A. pp. 213-227.
18. Gallun, R. L. (1977) Genetic basis of Hessian fly epidemics. Ann. New York Acad. Sci. 287 : 223-229.
19. Harrington, C. D. (1945) Biological races of the pea aphid. J. Econ. Ent. 38 : 12-22.
20. Harvey, T. L. and H. L. Hackerott (1979) Recognition of a greenbug biotype injurious to sorghum. J. Econ. Ent. 62 : 776-779.

21. Heinrichs, E. A. and S. L. Valencia (1978) Contact toxity of insecticides to the three biotypes of brown planthopper. IRRN 3(3): 19-20.
22. Iman, M. (1978) *Resistance of Rice Varieties to Biotype 2 of the Brown Planthopper, Nilaparvata lugens (Stål).* Ph D thesis. Univ. Philippines, 110 pp.
23. IRRI (1976) IRRI Ann. Rep. 1975, 479 pp.
24. IRRI (1977) IRRI Ann. Rep. 1976, 418 pp.
25. IRRI (1978) IRRI Ann. Rep. 1977, 548 pp.
26. Iwata, T. (1963) Ecological studies on the local variations in the seasonal history of the rice stem maggot, *Chlorops oryzae* Matsumura. Bull. Hokuriku Agr. Exp. Stat. No. 4: 109-189 (In Japanese with English summary).
27. Karim, A. N. M. R. (1975) *Resistance to the Brown Planthopper, Nilaparvata lugens (Stål) in Rice Varieties.* MS thesis. Univ. Philippines, 131 pp.
28. Keep, E. and R. L. Knight (1967) A new gene from *Rubus occidentalis* L. for resistance to strains 1, 2, and 3 of the rubus aphid, *Amphorophora rubi* Kalt. Euphytica 16: 209-214.
29. Lakshminarayana, A. and G. S. Khush (1977) New genes for resistance to the brown planthopper in rice. Crop. Sci. 17: 96-100.
30. Lammerink, J. (1968) A new biotype of cabbage aphid (*Brevicoryne brassicae* (L.)) on aphid resistant rape (*Brassica napus* L.). N. Z. J. Agr. Res. 11: 341-344.
31. Lichtwardt, E. T. (1956) Genetics of DDT-resistance of an inbred line of houseflies. J. Hered. 47: 11-16.
32. Lichtwardt, E. T., W. N. Bruce and G. C. Decker (1955) Notes on the inbreeding of house flies. J. Econ. Ent. 48: 301-303.
33. Ling, K. C., E. R. Tiongco and V. M. Aguiero (1978) Rice ragged stunt, a new virus disease. Pl. Dis. Rep. 62: 701-705.
34. Liquido, N. J. (1978) *Morphology, Cytology and Breeding Behavior of Nilaparvata lugens (Stål) (Delphacidae, Homoptera).* MS thesis, Univ. Philippines, 89 pp.
35. Lowe, H. J. B. (1974) Intraspecific variation of *Myzus persicae* on sugar beet (*Beta vulgaris*). Ann. Appl. Biol. 78: 15-26.
36. Müller, F. P. (1962) Biotypen und Unterarten des "Erbsenlaus" *Acyrthosiphon pisum* Harris. Z. PflKrankh. PflPath. PflSchutz. 69: 129-136.
37. Müller, F. P. (1966) Schädliche Blattläuse in den Tropen und Subtropten unter besonderer Berücksichtigung von Rassendifferenzierungen. Z. Angew. Ent. 58: 76-82.
38. Müller, F. P. (1969) Eine bionmische Rasse von *Aphis nasturtii* Kaltenbach, welche die Kartoffel meidet. Arch PflSchutz 5: 179-188.
39. Markkula, M. and K. Roukka (1970) Resistance of plants to the pea aphid, *Acyrthosiphon pisum* Harris (Hom., Aphidiae). I. Fecundity of the biotypes on different host plants. Ann. Agr. Fenn. 9: 127-132.
40. Meier, W. (1964) Uber einen Caudalhaarindex zur Charkterisierung von Klonen der Erbsenblattlaus *Acyrthosiphon pisum* Harris. Mitt. Schwiz. Ent. Ges. 37: 1-41.
41. Nielson, M. W. and H. Don (1974) Interaction between biotypes of the spotted alfalfa aphid and resistance in alfalfa. J. Econ. Ent. 67: 368-370.
42. Nielson, M. W., H. Don, M. H. Schonhorst, W. F. Lehman and V. L. Marble (1970) Biotypes of the spotted alfalfa aphid in Western United States. J. Econ. Ent. 63: 1822-1825.
43. Paguia, P., M. D. Pathak and E. A. Heinrichs (1980) Honeydew excretion measurement techniques for determining differential feeding activity of biotypes of *Nilaparvata lugens*

on rice varieties. J. Econ. Ent. 73 : 35-40.
44. Painter, R. H. (1951) *Insect Resistance in Crop Plants.* MacMillan, New York, 520 pp.
45. Phillips, P. A. and M. M. Barnes (1975) Host race formation among sympatric apple, walnut, and plum populations of the codling moth, *Laspeyresia pomonella.* Ann. Ent. Soc. Am. 68 : 1053-1060.
46. Pathak, M. D., C. H. Cheng and M. E. Fortuno (1969) Resistance of *Nephotettix impicticeps* and *Nilaparvata lugens* in varieties of rice. Nature (Lond.) 223 : 502-504.
47. Pathak, M. D. and R. H. Painter (1958) Differential amounts of material taken up by four biotypes of corn leaf aphids from resistant and susceptible sorghums. Ann. Ent. Soc. Am. 51 : 250-254.
48. Pathak, M. D. and R. H. Painter (1959) Geographical destribution of the four biotypes of corn leaf aphid, *Rhopalosiphum maidis* (Fitch), in Kansas. Kansas Acad. Sci. Trans. 62(1) : 1-8.
49. Rivera, C. T., S. H. Ou and T. T. Iida (1966) Grassy stunt disease of rice and its transmission by the planthopper *Nilaparvata lugens* Stål. Pl. Dis. Rep. 50 : 453-456.
50. Sen Gupta, G. C. and P. W. Miles (1975) Studies on the susceptibility of varieties of apple to the feeding of two strains of woolly aphis (Homoptera) in relation to the chemical content of the tissues of the host. Aust. J. Agr. Res. 26 : 157-168.
51. Shimura, I. (1972) Studies on the breeding of chestnut, *Castanea* spp. II Parasitic variation in the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu. Bull. Hort. Res. Stat. A-11 : 1-13 (In Japanese with English summary).
52. Smith, S. G. and B. A. Sugden (1969) Host trees and breeding sites of native North American *Pissodes* bark weevils, with a note on synonymy. Ann. Ent. Soc. Am. 62 : 146-148.
53. Sōgawa, K. (1970) Studies on feeding habits of the brown planthopper. I. Effects of nitrogen-deficiency of host plant on insect feeding. Jap. J. Appl. Ent. Zool. 14 : 101-106 (In Japanese with English summary).
54. Sōgawa, K. and C. H. Cheng (1979) Economic thresholds, nature of damage, and losses caused by the brown planthopper. See Ref. 11, pp. 125-142.
55. Sōgawa, K. and M. D. Pathak (1970) Mechanism of brown planthopper resistance in Mudgo variety of rice (Hemiptera : Delphacidae). Appl. Ent. Zool. 5 : 145-158.
56. Sona, Jr., O. (1978) Biotype L, ninth biotype of the Hessian fly. J. Econ. Ent. 71 : 458-460.
57. Sona, Jr., O. and R. L. Gallun (1973) Purification of race B and C of the Hessian fly by genetic manipulation. Ann. Ent. Soc. Am. 66 : 1065-1070.
58. Sparks, A. N., H. C. Chiang, A. J. Keaster, M. L. Fairchild and T. A. Brindley (1966) Field studies of the corn borer biotypes in the Midwest. J. Econ. Ent. 59 : 922-928.
59. Stevenson, A. B. (1970) Strains of the grape phylloxera in Ontario with different effects on the foliage of certain grape cultivars. J. Econ. Ent. 63 : 135-138.
60. Vandersar, T. J. D., J. H. Borden and J. A. McLean (1977) Host preference of *Pissodes strobi* Peck (Coleoptera : Curculionidae) reared from three native hosts. J. Chem. Ecol. 3 : 377-389.
61. Van Emden, H. F., V. F. Eastop, R. D. Hughes and M. J. Way (1969) The ecology of *Myzus persicae.* Ann. Rev. Ent. 14 : 197-270.
62. Varca, A. S. and R. Feuer (1976) The brown planthopper and its biotypes in the Philippines. Pl. Prot. News 5(1) : 1-4.
63. Wood, Jr., E. A. (1961) Biological studies of a new greenbug biotype. J. Econ. Ent. 54 :

71-1173.

64. Wood, Jr., E. A., H. L. Chada and P. N. Saxena (1969) Reaction of small grains and grain sorghum to three greenbug biotypes. Prog. Rep. P-618, Oklahoma Agr. Exp. Stat. 7 pp.

65. Yushima, K. (1968) Separation and detection of esterases by the agar gel electrophorasis. Shokubutsu-boeki 22 : 71-74 (In Japanese).

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