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Biotypes of the Brown Planthopper, *Nilaparvara lugens* (Stål)

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벼멸구의 生態型

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ABSTRACT

The brown planthopper, *N. lugens* (Stål), has become a serious pest of rice in tropical Asia during the last decade. At high pest density, its feeding damage causes 'hopperburn' or complete wilting and drying of the rice plant. It also transmits grassy and ragged stunt virus diseases. The estimated losses caused by the pest in tropical Asia exceed US$ 300 *millions*.

While cultivation of resistant rice varieties has proved to be highly effective against the pest, their long-term stability is threatened because of the evolution of prolific biotypes which can destroy these varieties. At present, identification of biotypes is based principally on the differential reactions of host rice varieties to the pest and on host-mediated behavioral and physiological responses of the pest. Recent findings of morphological differences in adult rostrum, legs, and antennae, body parts that possess receptors for host plant location and discrimination, and cytological differences in *N. lugens* populations maintained as stock cultures strongly complement other biotype studies.

So far, three *N. lugens* biotypes have been identified in the Philippines. Biotype 1 can survive on and damage varieties that do not carry and genes for resistance, while Biotype 2 survives on resistant varieties carrying *Bph 1* gene and Biotype 3 on varieties carrying gene *bph 2*. However, none of these biotypes can survive on varieties with genes *Bph 3* or *bph 4*.

Several varieties which are resistant in the Philippines are susceptible in India and Sri Lanka as the *South Asian biotypes* of *N. lugens* are more virulent than *Southeast Asian biotypes*. To monitor the pest biotypes in different geographical regions and to identify new sources of resistance, an International Brown Planthopper Nursery has been established in many cooperating countries.

The evolution of biotypes is an exceedingly complex process which is governed by the interactions of genetic and biological factors of the pest populations and the genetic makeup of the cultivated varieties. While the strategy for sequential release of varieties with major resistance genes has been fairly successful so far, the monegenic resistance of these varieties

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makes them vulnerable to the development of the pest biotypes. Therefore, present breeding endeavors envisage utilizing both major and minor resistance genes for effective control of the pest.

INTRODUCTION

THE BROWN PLANTHOPPER (BPH), *Nilaparvata lugens* (Stål), is a prolific and genetically plastic species. Although catastrophic BPH outbreaks had periodically been recorded in the temperate rice-growing countries of Asia, such as Korea (Okamoto, 1924) and Japan (Suenga and Nakatsuka, 1958), it was considered a pest of only minor importance in tropical Asia until the last decade. Its rise to prominence as a key pest in tropical Asia coincided with the introduction of high yielding rice varieties grown under improved irrigation and high rates of nitrogenous fertilizer application. Crop intensification as a result of the availability of short maturity cultivars further accentuated the BPH problem. Large-scale damage by these pest in India, Indonesia, the Philippines, and Sri Lanka was reported in the early 1970s (Dyck and Thomas, 1979). In addition, infestations of varying severity are now commonly observed in Australia, Bangladesh, Brunei, China, Fiji, Korea, Malaysia, Papua New Guinea, Solomon Islands, Taiwan, Thailand, and Vietnam.

The pest feeds directly on the growing plant, reducing yield. In high numbers it causes a complete wilting or drying of the crop-a condition called "hopperburn". The insect also transmits grassy stunt and ragged stunt virus diseases (Ling, 1967; Ling et al., 1978). The estimated losses due to BPH-transmitted diseases exceed US $ 300 million (Dyck and Thomas, 1979).

The magnitude of the pest outbreaks in tropical Asia prompted rice scientists to develop resistant varieties as the most practical solution to the BPH problem in the developing countries. Concerted efforts to evalutate and utilize BPH-resistance in the available germplasm began in 1966 at the International Rice Research Institute (IRRI) in the Philippines. About a year later, high levels of resistance were discovered in the variety Mudgo (Pathak et al., 1969) and a few plants of IR532 (Peta[3]/TNI/TKM6) (IRRI, 1967). Despite this early success, breeding for BPH resistance has remained a challenging task. More than 50,000 rice varieties and accessions have been evaluated at IRRI in a continual search for new scurces of resistance to deal with the problem of BPH biotypes. The term

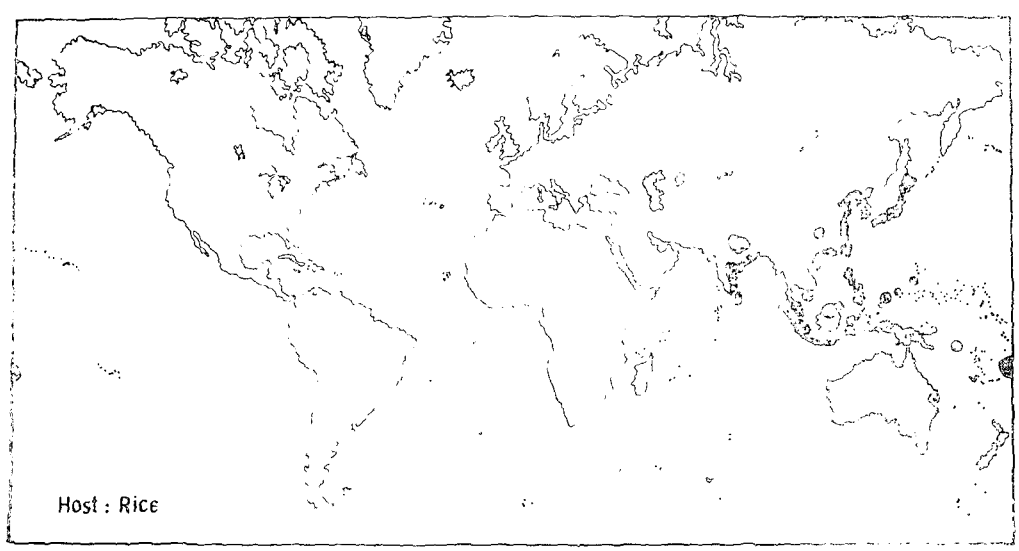

Fig. 1. Distribution of the brown planthopper, *Nilaparvata lugens* (Stål), shown by blackened areas (source: R.C. Saxena, IRRI).

biotype is used herein in the sense of Gallun and Khush (1980) and refers to pest populations which differ in their ability to infect rice varieties with specific major genes for resistance.

OCCURRENCE AND EVOLUTION OF BIOTYPES

Diversification and specialization of an insect pest species into biotypes enables it to keep pace with the evolution of the defenses of the host plant through either natural selection or by man's mani pulation of the host plant's genome through conventional or innovative breeding techniques. The threat of *N. lugens* biotypes to the stability of resistant varieties is particularly serious bebause of its marked gentic plasticity and wide range of distribution (Fig. 1). Its small size, short life cycle, short generation time, and high fecundity results in high reproductive rates which permit dramatic changes in population sizes and rapid differentiation of populations under dissimilar selection regimes.

Studies of the possible occurrence and evolution of BPH biotypes first began at IRRI in 1971, when field populations were collected from different places in Luzon province in the Philippines and their survival rates tested on the susceptible TN1 and the resistant Mudgo rice varieties(IRRI, 1972). Repeated collecting and rearing of field populations on resistant plants culminated in the identification of three distinct, *Sympatric* BPH populations (*Southeast Asian biotypes*) which were designated as Biotypes 1, 2, and 3 (Fig. 2) (IRRI, 1976; Pathak and Sayena, 1980). Biotype 1 could infest only those varieties which appear to lack genes for resistance, while Biotype 2 was able to survive on resistant varieties carrying gene *Bph 1* and Biotype 3 on varieties carrying gene *bph 2*. However, none of these biotypes can survive on varieties with

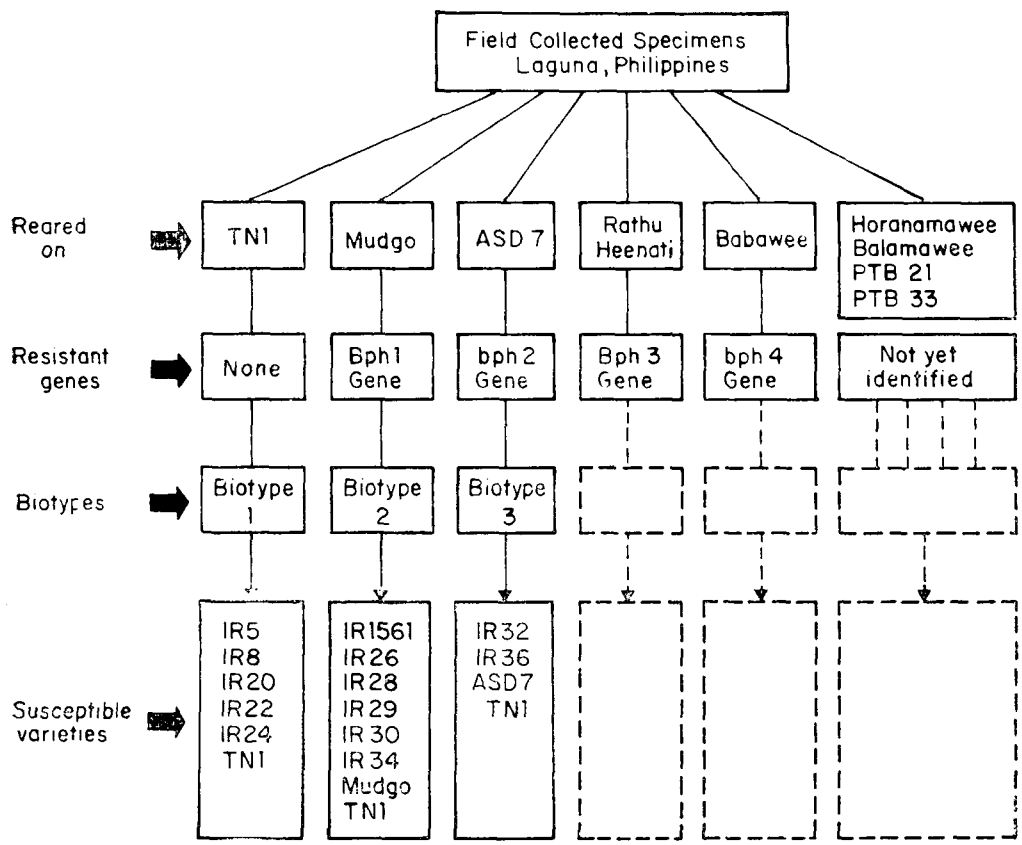

Fig. 2. Schematic representation of development of sympatric Biotypes 1, 2, and 3 (Pathak and Saxena 1980).

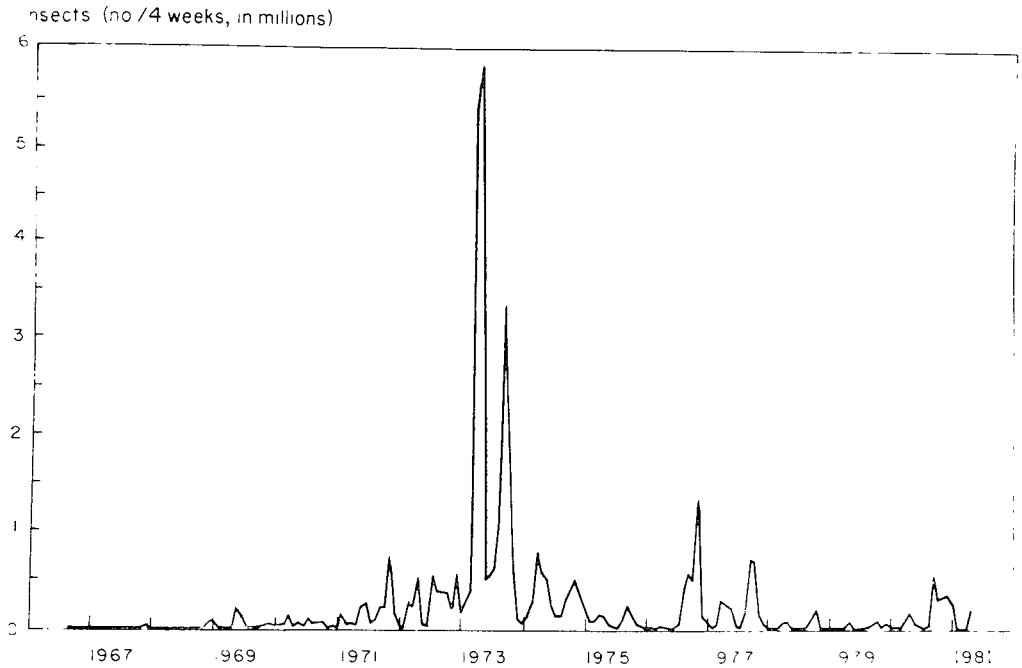

Fig. 3. Average number of *N. lugens* macropters attracted to 3 light traps at IRRI (V.A. Dyck, unpublished data). Intensive cultivation of resistant varieties is considered a major factor in the decline of *N. lugens* numbers since mid-1973

Table 1. Grouping of *N. lugens* biotypes based on differential reaction of varieties in South and Southeast Asia.[1]

Biotype	Varietal Reaction (R, MR, S)[2]
Southeast Asia	
Biotype 1—Philippines, China, Japan, Korea, Malaysia, Taiwan, Thailand	R-MR: IR26, ASD7, Rathu Heenati, Babawee, Ptb 33 S: ARC 10550, TN1
Biotype 2—Philippines, Solomon Islands, Vietnam	R: ASD7, Rathu Heenati, Babawee, Ptb 33 S: IR26, ARC 10550, TN1
Biotype 3—Philippines, Taiwan	R: IR26, Rathu Heenati, Babawee, Ptb 33 S: ASD7, ARC 10550, TN1
South Asia	
Bangladesh, Hyderabad (India)	R-MR: Rathu Heenati, Babawee, Ptb 33. ARC 10550 S: IR26, ASD7, TN1
Coimbatore (India)	R: Babawee, Ptb 33, ARC 10550 S: Rathu Heenati, IR26, ASD7, TN1
Pantnagar (India)	S: IR26, ASD7, Rathu Heenati, Babawee, Ptb 33, ARC 10550, TN1

[1]Revised from IRPS No. 72, Feb. 1982.
[2]R = resistant, MR = moderately resistant, S = susceptible.

resistance genes *Bph 3* and *bph 4* and on Ptb 33 with 2 unidentified genes. These genes are being bred into many new varieties with improved plant type.

The first released BPH-resistant variety, IR26, was highly successful against the general population (predominantly Biotype 1) in the Philippines in 1973~75 (Fig. 3) (Pathak and Khush, 1979) and in Indonesia in 1974-76 (Harahap, 1979). However, this popular variety soon succumbed to a new selection (Biotype 2) of the pest in the Philippines (Varca and Feuer, 1976), Indonesia (Harahap, 1979), and Vietnam (Huynh, 1977). Reports of widespread hopperburn suggested a shift in the BPH population because of the extreme selection pressure exerted by intensive planting of IR26. It also indicated that BPH populations were fairly flexible in their response to rice varieties.

About the same time, in distant geographical regions, Kerala, India, and the neighboring Sri Lanka, IR26 in field and greenhouse testing was found to be susceptible (Pathak and Khush, 1979). This was the first evidence of an *allopatric* virulent population (*South Asian Biotype*) of the pest(Table I). To monitor other allopatric biotypes and to identify new resistant material, an International Brown Planthopper Nursery (IRBPHN) has been established in many countries through the International Rice Testing Program (IRTP) coordinated by IRRI. Based on recent IRBPHN results, additional virulent biotypes of *N. lugens* are suspected to exist in various rice growing parts of the Indian sub-continent (Varma et al., 1979; IRRI, 1982). Several varieties which are resistant in the Philippines are susceptible to some of these biotypes in greenhouse and field screening in India. The richness of sources of resistance in rices of India and Sri Lanka and the simultaneous occurrence of virulent biotypes indicates a prolonged coevolution between BPH and the rice host in that geographic region.

In Southeast Asian countries, such as Indonesia, Philippines, Vietnam, etc., where Biotype 3 is fortunately not yet widespread, varieties with *bph 2* gene (e.g. IR36) have been cultivated widely and successfully since about 1976. However, the threat of selection and the spread of the pest biotypes can not be ignored. Recently, Pathak and Heinrichs (1982) demonstrated that selection of Biotype 2 and 3 populations from the original *N. lugens* population maintained on the susceptible TNI variety could be attained in about 8 generations by exposing the latter to resistant varieties. The rapid adaptations of this insect provides evidence that biotype segregation can occur within relatively few generations.

Recently, a new population of *N. lugens* has been found to occur on the common weed, *Leersia hexandra* on the IRRI experimental farm and in some other locations in Luzon province (Heinrichs and Medrano, personal communication). While this 'biotype' can infest and kill *Leersia* host plants, it has failed to survive on any of the rice varieties tested so far.

The evolution of biotypes is an exceedingly complex process which is governed by interactions of the genetic and biological characteristics of pest populations and the extent of cultivation of resistant varieties. The mode of interaction between the pest and the host systems is essentially genetic in nature. In addition, the genetic factors may involve the dominance and initial frequency of genes that confer the ability on the pest to overcome the host plant's resistance.

Bush (1974) presented two plausible explanations for sympatric speciation through host race or biotype formation: (1) The host system induces an abrupt change on the insect's survival locus (loci) conferring an advantage of fitness on the surviving genotypes; (2) The insect population is pre-adapted and the best fitted genotypes are expressed depending on the existing environmental conditions. Also the possibility of the development of pest biotype through mutation (Nielson and Don, 1974; Harris 1975; Pathak, 1975) or their spread through migration should not be excluded. It seems, however more reasonable to assume that host specific biotypes develop through natural selection acting upon the genetic variation within the species.

In the case of *N. lugens*, it has been pointed out that its natural populations usually include small proportions of preexisting biotypic variants which upon selection become the forerunners of new

biotypes (IRRI, 1976).

VARIATIONS AMONG BIOTYPES

Relative susceptibility or resistance of rice varieties to BPH is determined by an interaction of the insect's responses to the rice plant and the effects of the latter's physical and chemical stimuli on the insect's behavior and physiology. Variations in the pest's populations or biotypes can be monitored through both differential varietal reactions as well as the pest's responses. Also, recent investigations at IRRI have demonstrated the existence of subtle electrophoretic, morphological, and cytological variations among BPH biotypes.

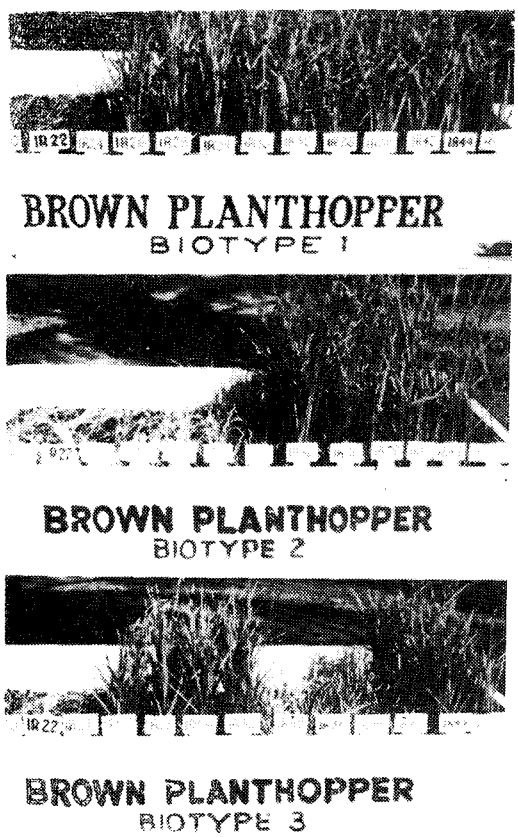

Fig. 4. Differential varietal reactions to Biotypes 1, 2, and 3. Most of the recent IRRI varieties are resistant to Biotypes 1 and 2. Biotype 3 attacks plants resistant to Biotype 2. None of these biotypes can attack varieties with *Bph 3* or *bph 4* gene.

Differential Varietal Reactions

Differential host varieties are most commonly used to detect biotypes of insect pests. Both sympatric and allopatric biotypes of *N. lugens* have been monitored on the basis of differential varietal reactions studied in greenhouse screenings and in field plantings of test nurseries at many locations (Oka, 1978; Seshu and Kauffman, 1980; IRRI, 1982). Varieties susceptible to a particular biotype are killed upon infestation with nymphs or adults while those resistant remain green and healthy (Fig. 4).

Host-Mediated Differential Responses

Biotypes can also be separated from each other by their differential responses to plants of known genotypes. Although BPH does not show differences in alighting upon susceptible or resistants, its feeding activity is not sustained on resistant varieties (Saxena and Pathak, 1977). Therefore, measurements of duration and quantity of ingested food on susceptible and resistant genotypes are good indicators for biotype identification despite individual variations in food intake (Fig. 5).

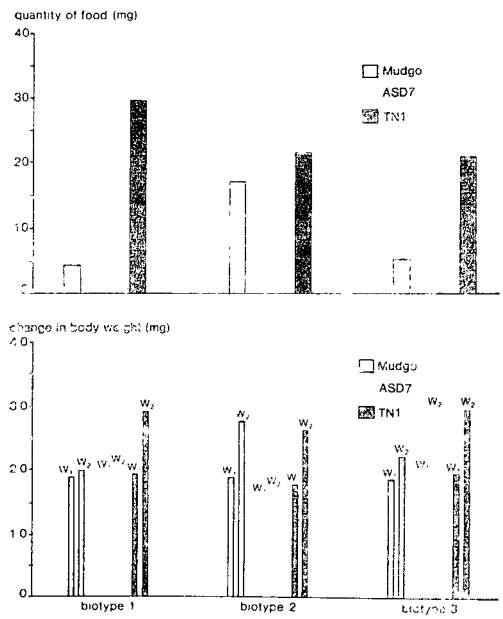

Fig. 5. Quantity of food ingested per brachypterous female in 24 h and change in body weight of female in 24 h of three *N. lugens* biotypes allowed to feed on Mudgo, ASD7, and TN1 rice varieties. W_1 initial weight, W_2 final weight after 24 h (Saxena and Pathak, 1977).

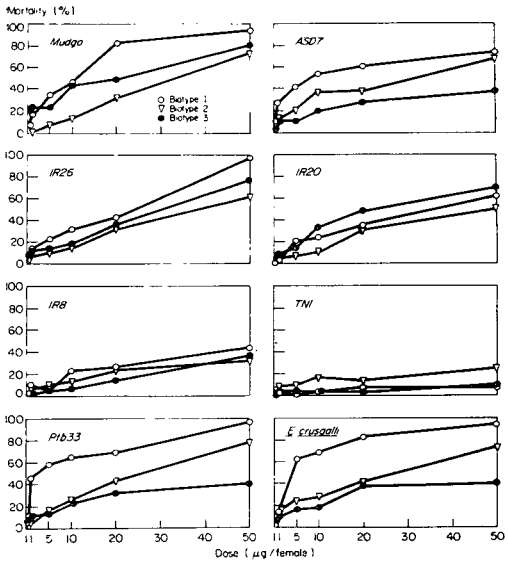

Fig. 6. Mortality of brachypterous females of *N. lugens* biotypes 24 h after topical application of steam distillate extracts of different rice varieties and barnyard grass. IRRI, 1979.

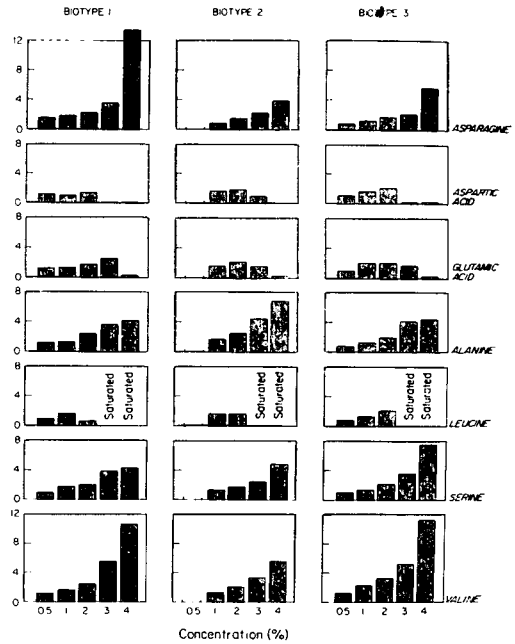

Fig. 7. Relative intake of seven amino acid solutions of different concentrations by three biotypes of *N. lugens*.

Although a number of simple techniques have been devised and used (Paguia et al., 1980), the use of the parafilm sachet, originally developed by Saxena and Pathak (1977) and later modified by Pathak et al. (1982), is the most reliable method for the collection and quantitative determination of honeydew excreted by BPH feeding on rice. Food intake in BPH is related to the amount of honeydew excreted. The insect's weight gain is smaller on resistant varieties as against susceptible varieties and is related to the amount of sap sucked. A biotype incapable of feeding adequately on a resistant variety, therefore, fails to establish itself in as large numbers as on a susceptible variety.

BPH biotypes also show distin tive responses to allelochemics and nutritional factors of rice varieties. Thus, in orientation tests, Biotype 1 was found to be attracted to the odor emanating from the steam distillate extract of the susceptible TNl variety, but repelled by the odor of the resistant Mudgo variety(Saxena and Pathak, 1977). On the other hand, the distillate of Mudgo attracted Biolype 2, and the distillate of ASD7 attracted Biotype 3. Mudgo is susceptble to Biotype 2, and ASD7 is susceptible to Biotype 3.

BPH Biotypes 1, 2, and 3 showed distinctive mortality patterns when topically treated with the steam distillate extracts, principally essential oils which comprise the plant's volatile components and with which insects come in contact upon arrival (IRRI, 1979) (Fig. 6). Thus, Biotype 1 was highly snsceptible to low doses of extracts of all resistant varieties and the barnyard grass, but few insects died at even higher doses of the susceptible IR8 and TNl varieties. Biotype 2 suffered high mortality with extracts of barnyard grass, Ptb 33, and ASD7. Biotype 2 mortality was low with IR8 and TNl extracts and also with low doses of Mudgo extract. Biotype 3 insects suffered relatively higher mortality in treatments with extracts of Mudgo, IR26 and IR20 than in those with ASD7, IR8, and TNl extracts. The toxic effect of Ptb 33 and barnyard grass extracts, however, were not as pronounced as those on other biotypes.

BPH biotypes 1, 2, and 3 indicated a strong chemosensory specificity in bioassays with seven common rice amino acids-alanine, asparagine, aspartic acid, glutamic acid, leucine, serine, and valine (Fig. 7). For Biotype 1, asparagine and valine

were most phagostimulatory at 4% concentration while alanine and serine were moderately phagostimulant. For Biotype 2, alanine at 4% concentration was most phagostimulatory, while valine, serine, and asparagine were moderately phagostimulatory in that order. For Biotype 3, valine and serine were most phagostimulatory followed by asparagine and alanine. Leucine was more or less inert for the three biotypes while aspartic and glutamic acids were inhibitory at higher concentrations. The content of phagostimulatory amino acids specific to each biotype were correspondingly higher in their respective preferred rice variety.

Electrophoretic Variations

Esterase polymorphism in males of laboratory colonies of the three biotypes and of the field population was determined by agar gel eletrophoresis on individual insect homogenate (Sogawa, 1978a). Six electrophoretic phenotypes were detected and designated as A to F. The zymogram of each type was composed of 3 to 6 esterase bands with different mobility towards the anode side. Type C was common to all biotypes and the field population. Type E was also invariably detected in all biotypes, although with less frequency than type C. Type D with esterase E_3 band appeared almost exclusively in Biotype 2, demonstrating that this biotype could be differentiated from the other biotypes. However, further, studies on genetic system that controls the estrase polymorphism are needed.

Morphological Variations

Bey-Bienko (1958) noted that in many organisms, changes in ecological and physiological traits of the species are frequently followed by subtle changes in morphological characteristics. Several workers(Liquido, 1978; Sogawa, 1978b; Claridge and Den Hollander, 1980) also attempted, but failed, to detect any significant morphological differences among biotypes of *N. lugens*. These workers examined such body characters as dimensions of the head capsule, hind tibia, tegmen, male and female genitalia, the number of teeth on tibial spurs, and the number of spines on the phallus and the hind basitarsus. Some variation was observed among biotypes in the frequency distribution of numbers of spines on the hind basitarsus of adults, but occasionally a leg on one side had more spines than others. All these characters were, therefore, nonbiotype specific.

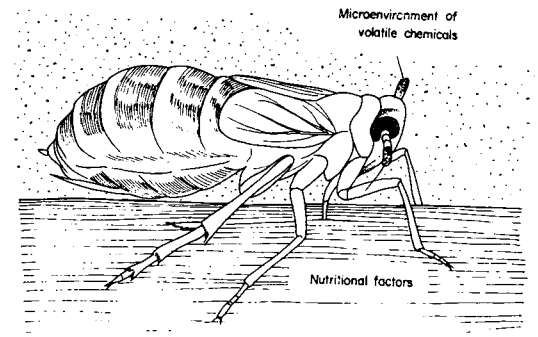

Fig. 8. Rostral, leg, and antennal receptors play an important role in host location and discrimination by *N. lugens* (Saxena and Rueda, in press).

Considering morphology as the end product of behavioral and physiological activities initiated by the genome and modified by the environment, Saxena and Rueda (in press) established a morphological basis for the identification of BPH biotypes. They made in-depth evaluations of morphological and morphometric differences among popultions of Biotypes 1, 2, and 3 maintained as stock cultures at IRRI for several years, emphasizing on body parts possessing receptors for host plant discrimin-

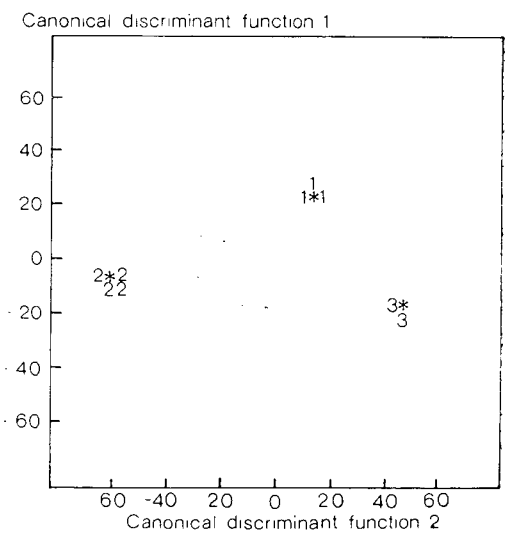

Fig. 9. Discriminant scores of Biotypes 1, 2, and 3 of *N. lugens* based on adult rostral, leg, and antennal characters of brachypterous females (Saxena and Rueda, in press).

Table 2. Predicted group membership of Biotypes 1, 2, and of *N. lugens* based on rostral, leg, and antennal characters of macropterous and bracypterous males and femals (Saxena and Reuda, in press).

Data set	Group	Adult character[1] description	Sample[2] size (no.)	Variables entered (no.)	Variables in the function (no.)	Functions (no.)	Variance (%) accounted for by Function 1	Variance (%) accounted for by Function 2	Group (%) cases correctly identified
I	1	Rostral, MAC♂	30	16	9	2	94	6	97
	2	Rostral, MAC♀	30	16	9	2	71	29	90
	3	Rostral, BRAC♂	30	16	3	2	79	21	73
	4	Rostral, BRAC♀	30	16	9	2	78	22	87
II	(	Leg, MAC♂	30	57	25	2	93	7	100
	2	Leg, MAC♀	30	57	25	2	85	15	100
	3	Leg, BRAC♂	30	52	25	2	94	6	100
	4	Leg, BRAC♀	30	57	24	2	99	1	100
III	1	Antennal, MAC♂	30	36	19	2	67	33	100
	2	Antennal, MAC♀	30	36	17	2	98	2	100
	3	Antennal, BRAC♂	30	36	24	2	87	13	100
	4	Antennal, BRAC♀	30	36	22	2	79	21	100
IV	1	Rostral+leg+ antennal, MAC♂	30	109	23	2	80	20	100
	2	Rostral+leg+ antennal, MAC♀	30	109	23	2	69	31	100
	3	Rostral+leg+ antennal, BRAC♂	30	109	23	2	86	14	100
	4	Rostral+leg+ antennal, BRAC♀	30	109	23	2	86	14	100

[1] MAC=macropterous, BRAC=brachypterous.
[2] Each group consisted of 10 individuals each of Biotypes 1, 2, and 3.

ation (Fig. 8). More than 100 morphological characters of rostrum, including mandibular stylets, legs and antennae were measured and evaluated. Characters were examined separately in both sexes and morphs, i.e., macropterous male, macropterous female, brachypterous male, and brachypterous female.

Multiple discriminant analysis using stepwise selection through Wilk's specification indicated distinct segregation of the three biotypes. The characters of the rostrum, legs and antennae common to both sexes and their respective morphs contributed to the segregation of biotypes. The scatter diagrams based on computed discriminant scores of the three biotypes strongly revealed a high degree of segregation, thus, classifying them as distinct populations (Fig. 9). The hoppers classified on leg and antennal characters exhibited a 100% probability of correct morphological identification of the three BPH biotypes (Table 2). Only the rostral characters showed a slightly lower percentage of correct biotype identification-about 73% for brachypterous males, 87% for brachypterous females, 97% for macropterous males, and 90% for macropterous females.

These findings for the first time showed that considerable genetic varieties may be concealed in '*apparently*' morphologically alike individuals of and populations of *N. lugens* biotypes. These criteria are now being used in evaluating populations of BPH biotypes from other geographical areas. The technique of morphological evaluation of insect biotypes also provides an additional tool for study of speciation and confers the added advantage that variation can be monitord even in preserved specimens, obviating the need to transport live materials.

Table 3. Variations in nuclear and chromosome measurements of *N. lugens* Biotypes 1, 2, and 3 during prophase I (Saxena and Barrion, unpublished). IRRI, 1981-82.

Prophase I (substages)	Biotype 1	Biotype 2	Biotype 3
Differences in absolute mean length (*aml*), absolute mean width (*amw*) and relative mean length (*rml*)			
Leptonema (Nuclei: *aml* and *amw*)^{ns}	31.75μ and 26.50μ. highest	29.90μ and 24.00μ, lowest	30.50μ and 24.75μ, intermediate
Zygonema (Autosomes) (Sex chromosome)	no difference	no difference	no difference
Pachynema (Autosomes *rml*) (Sex chromosome *rml*)^{ns}	11.11mm, lowest 6.50mm, lowest	12.43mm, intermediate 9.00mm, highest	13.17mm, highest 7.00mm, intermediate
Diplonema (Autosomes *aml*)* (Sex chromosome *aml*)	5.92μ, lowest 7.50μ	8.08μ, highest 5.00μ	7.08μ, intermediate 5.00μ
Diakinesis (Chromosomes *aml*)†	4.35μ highest	3.77μ, intermediate	3.39μ, lowest

^{ns} not significant at 5% level by ANOVA and DMRT.

* Biotype 1 significantly different from Biotypes 2 and 3; Biotypes 2 and 3 not significantly different from each other at 5% level by ANOVA and DMRT.

† Biotype 1 significantly different from Biotype 3 but not from Biotype 2; Biotype 2 not significantly different from Biotype 3 at 5% level by ANOVA and DMRT.

Cytological variations

Chromosome number, morphology, and behavior have often been relied upon as complementary taxonomic indicators in a number of species complexes. The sex chromosomes are especially useful in cytotaxonomy because they may show from marked to subtle differences within a genus or species. Recent cytogenetic studies of the meiotic chromosomes of BPH biotype 1, 2, and 3 populations, maintained as stock culture at IRRI for several years, revealed that the first and second divisions of meiosis were reductional and equational, respectively, for all the components of the species' genome (Saxena and Barrion, 1982a, 1982b, unpublished information). The male diploid number (2n=30) consisted of 14 bivalent autosomal pairs and XY chromosomes. Thus, *N. lugens* has an XY sex determining mechanism, the males being heterogamtic (14+XY) or producing two types of secondary spermatocytes and the females homogametic (14+XX) or producing one type of secondary oocytes.

Salient variations in nuclear and chromosome measurements during the different substages of *prophase I* are shown in Table 3.

Chromosomal behavior during *metaphase I* in the primary spermatocytes of the three BPH biotypes has been studied only recently (Saxena and Barrion, 1982b; Saxena and Barrion, unpublished). It features as clumping or clustering of highly condensed and shortened autosomes at the equatorial portion of the reproductive cell and separation of the highly heterochromatic, unequally synapsed sex chromosomes from the autosomal grouping. The clumping together of autosomes is mainly due to intra and interchiasmatic matrices between the homologous bivalent chromosomes and among the tetrads or homologues. The following observations were noteworthy during metaphase I in the three biotypes:

1. The highest number of metaphase I cells were detected from Biotype 1 followed by Biotype 3; Biotype 2 had the least number of cells.
2. Whereas Biotypes 1 and 3 revealed two types of metaphase I cells, namely, cells with sex chromosomes isolated from autosomes and cells with both chromosome types grouped together, Biotype 2 showed only the first type of cells (Fig.10).
3. The average distance of the sex chromosomes from autosomal grouping was highest in Biotype 2, almost twice that of Biotype 1, while Biotype 3 ranked next.
4. More cells with combined autosomes and sex chromosomes were observed in Biotype 1 than

in Biotype 3. Intra and interchiasmatic connections were higher in Biotype 1 than in Biotype 3 homologues.

5. The sex chromosomes of the three biotypes varied in lengths and widths. Biotype 2 had the longest chromosomes, follwed by Biotype 3, and Biotype 1 had the shortest. In terms of the wid ths of the X and Y chromosomes, Biotype 2 had the widest girth, while Biotypes 1 and 3 had almost equal measurements.

During *anaphase I*, measurements of the chromosome clumps at the two poles of the cells indicated that the lengths and widths of chromosome groupings in Biotype 2 were significantly different from those of Biotype 1 but not from Biotype 3.

At *telophase I*, the groupings of chromosomes at two opposite poles of the cells in the three biotypes had almost equal measurements of chromosomal clumps.

The occurrence of chromosomal aberrations, such as loose pairings of paired homologues bivalents as well as fragmentations or chromosomal deletions were found to be more frequent among Biotype 1 individuals, followed by Biotype 3, and the least in Biotype 2.

GENETICS OF BIOTYPES

Genetic studies of *N. lugens* biotyes are far

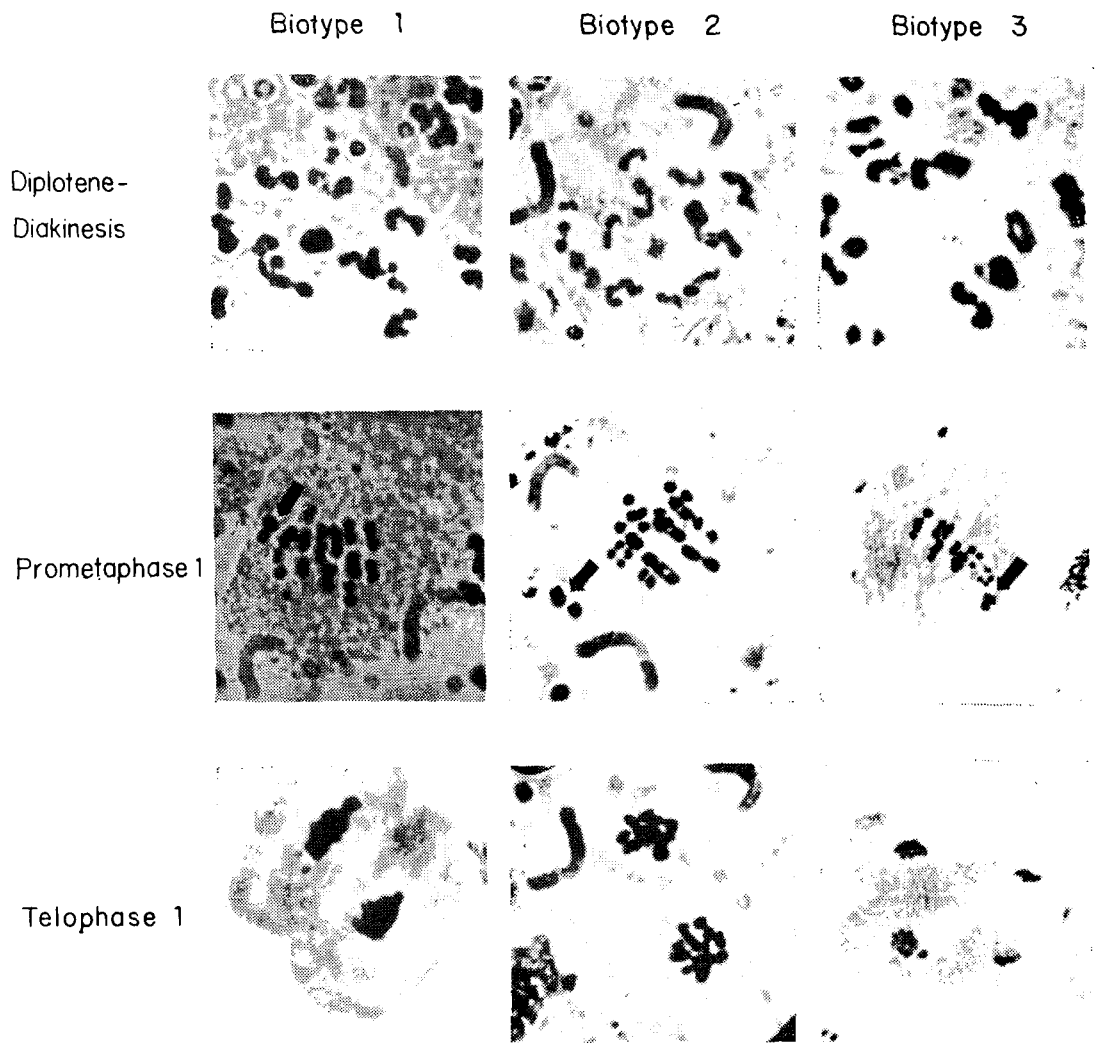

Fig. 10. First meiotic chromosomes of spermatocytes of *N. lugens* Biotypes 1, 2, and 3. Sex chromosome are indicated by arrows (Saxena and Barrion, unpublished).

from conclusive. Only preliminary investigations have been made into the inheritance of virulence of different BPH biotypes. Hybridization experiments conducted in Taiwan suggested that Biotype 2 was recessive to Biotype 1 while Biotype 3 was dominant over Biotype 1 on the basis of damage caused to seedlings of differential varieties by F1 hybrids (Cheng, 1975; Cheng and Chang, 1979). On the other hand, studies conducted at IRRI suggested that Biotype 1 was dominant over both Biotypes 2 and 3 and that Biotype 3 was dominant over Biotype 2 (IRRI, 1978). Recently, Sogawa (1981) indicated that biological traits (e.g. host preference, feeding behavior, growth and development, and fecundity) of Biotypes 2 and 3 were generally inherited in a recessive or intermediate manner when crossed with Biotype 1, but reports that Biotype 3 was dominant over Biotype 1 (Cheng, 1975; Cheng and Chang, 1979) and Biotype 2 (IRRI, 1978) were not confirmed. These observations suggested a gene-for-gene relationship between pest virulence and host plant resistance. However, hybridization results obtained by Den Hollander and Pathak(1081)were interpreted as indicative of a complex polygenic system of inheritance for virulence of Biotypes 1, 2, and 3.

POSSIBLE SOLUTIONS TO BIOTYPE PROBLEM

Despite the problem of biotypes,varietal resistance will continue to be the dominant factor in controlling BPH. Incorporation of BPH resistance has become an essential feature of the varietal improvement programs at both national and international levels and vast areas of rice are being planted to BPH-resistant varieties in Southeast Asia. However, because varieties with monogenic (major gene) resistance have short life spans, the future thrust will be to broaden the genetic base of resistance, both in terms of major and minor genes. IRRI has adopted several different strategies in breeding for resistance to BPH (Khush, 1979).

Sequential Release of Varieties with Major Genes

This strategy involves incorporation of a single major resistance gene into improved plant types and making them available to farmers for cultivation sequentially. Thus, varieties like IR26, IR28, IR29, and IR30, with *Bph1* gene were widely grown in the Philippines and Indonesia during 1973 ~76. When Biotype 2 capable of damaging these varieties became predominant in 1976, IR32, IR36, and IR38 with *bph 2* gene were released. Since then, these varieties have been grown extensively. IRRI has incorporated *Bph 3* and *Bph 4* genes into line with suitable agronomic background. Recently, variety IR56 with *Bph 3* gene has been distributed for cultivation in such problem areas as Mindanao in the Philippines where IR36 has reportedly been damaged severely by Biotype 3. As further new genes for resistance are identified, they will be incorporated into improved lines or varieties. It may also be possible to systematically rotate the same specific resistance genes provided only a particular biotype is widespread at a certain time and space. But this strategy has not been tested so far because new sources of BPH resistance are still being discovered.

Pyramiding the Major Genes

This strategy aims at pyramiding two or more major genes in the same improved variety. Because *Bph 1* and *bph 2* genes are closely linked, attempts to breed lines carrying both genes have not met success. However, *Bph3* and *bph 4* genes segregate independently of *Bph 1* and *bph 2*, and crosses have been made to combine *Bph 3*, *bph 2* and *Bph 3*, *Bph 1* and *bph4*, and *bph 2* and *bph 4*. Varieties with two major genes for resistance are expected to remain effective longer and may slow down the development of biotypes.

Multiline Varieties

This approach envisages incorporating several major genes into an isogenic background and then mixing these lines to form a multiline variety. This strategy was followed in breeding oats for crown rust resistance in Iowa, U.S.A. (Browning and Frey, 1969). However, the utility of this techniques in breeding for insect resistance remains largely untested, although some attempts have been made at IRRI for transferring the known major genes for BPH resistance into an isogenic background. The feasibility of this technique can be tested only

when appropriate materials become easily available and if there is imminent threat to the effectiveness of other strategies.

Horizontal Resistance

Breeders and entomologists are searching for ways of increasing the stability of resistance through the development of varieties having horizontal resistance. The concept of moderate resistance as a means of showing or preventing biotype selection is being studied and the possibility of incorporating minor genes for resistance into suitable plant type by a process of recurrent selection is being explored at IRRI. Several unimproved varieties and a few improved breeding lines with low levels of BPH resistance have been identified. For example, Utri Rajapan, a local Indonesian cultivar, has been found to be tolerant to all the three BPH biotypes at IRRI (Panda and Heinrichs, 1980). Crosses with such varieties should yield progenies with stable type of resistance, but developing improved germplasm with horizontal resistance is a slow process because of low heritability.

CONCLUSIONS

The threat of selection and spread of prolific *N. lugens* biotypes can not be ignored. Varieties with relatively more stable resistance will be needed to slow down the process of biotype development. While it took only about 2 years for Biotype 2 to become predominant and damage IR26(*Bph 1* gene) in the Philippines, the variety IR36 (*bph 2* gene) continues to be planted widely and successfully since its introduction in 1976. However, this success should be considered with a sense of caution. Recent reports of BPH damage to IR36 in Mindanao, Philippines, are indicative of a new shift in biotype popultion. The new variety IR56 (*Bph 3* gene) which has been made available to farmers for cultivation in that region is expected to provide a further lease of effective BPH control. This grace period should be utilized not only for developing new resistant varieties, but also aim at a better understanding of insect-plant interactions. This would require interdisciplinary studies of inheritance of rice plant's defense chemicals as well as further investigations into the pest's behavior, sensory and regulatory physiology, ecology, genetics, and sophisticated techniques of biotype identification. Pure line populations of *N. lugens* biotypes will be needed for these studies and for identifying new genetic sources of resistance in the world collection of the rice germplasm.

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