The significance of morphometric and fecundity differences between the 'biotypes' of the Brown Planthopper, *Nilaparvata lugens*

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Abstract

A morphometric analysis of biotypes 1, 2 and 3 from the International Rice Research Institute, Philippines, reared respectively on rice cultivars TN1, Mudgo and ASD7, showed significant differences, but some overlap between them. When the three biotypes were each reared for a single generation on TN1, morphometric differences were very greatly reduced and the distributions widely overlapped.

Biotypes 2 and 3 were significantly less fecund than biotype 1 when reared on their normal host varieties. When all were reared on TN1, biotype 3 showed a somewhat lower fecundity than did 1 and 2, but the difference was less than previously reported.

It is concluded that the evidence for the association of morphometric differences with virulence characteristics in *N. lugens* is not proved. Equally there is no evidence that morphometric data may be used to identify field populations with distinct patterns of virulence.

Introduction

Populations of the Brown Planthopper, *Nilaparvata lugens* (Stål), which are virulent to different resistant varieties of rice have been termed biotypes, and given numbers (IRRI, 1976). At the International Rice Research Institute (IRRI), Philippines, populations of biotypes 2 and 3, which were able to overcome the phenotypic effects of *Bph* 1 and *bph* 2 genes respectively, were originally selected from a biotype 1 (or field) population, which was virulent only to varieties incorporating no known genes for resistance (Pathak & Khush, 1979). These three biotype populations have been maintained in culture at IRRI since 1974 and consistently reared respectively on TN1, Mudgo and ASD7. They have been widely used to screen rice varieties from other regions in the search for new sources of resistance (Pathak & Khush, 1979). Field populations with similar patterns of virulence have been designated as the same biotype.

Several studies on the IRRI biotypes have revealed that, although the populations differ on average in: (1) survival, (2) time for development to adult and (3) ability to feed on the different resistant varieties (IRRI, 1979; Sogawa, 1981a, b), there is wide variation within each population, with considerable overlap between them (Claridge & Den Hollander, 1980). Crosses between the biotypes revealed that virulence was probably under polygenic control (Den Hollander & Pathak, 1981; Sogawa, 1981b). Also, by laboratory selection experiments, different workers have shown that it may require less than ten generations to transmute one 'biotype' to another capable of attacking a different resistant variety (Varca & Feuer, 1976; Kaneda & Kisimoto 1977; Cheng, 1977; Pathak & Heinrichs, 1982; Claridge & Den Hollander, 1982).

No evidence has been found for any barriers to random mating between the IRRI biotypes and we have concluded that these 'biotypes' represent rather arbitrary divisions of an extremely variable spe-

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cies and that the use of the term, and especially the naming and numbering of biotypes, may be positively misleading in attempts to understand the evolution of virulence patterns in this species (Claridge & Den Hollander, 1980, 1982, 1983).

However, the apparent clear cut differential survival of rice varieties when exposed to large populations of the insects in screening tests (Choi, 1979; Seshu & Kauffman, 1980; IRRI, 1976) has reinforced the belief of some workers that the biotypes are clearly separate and discrete entities which may be identified in different geographical regions. This belief and the desire for an easy method of identifying virulent populations has spurred efforts to find criteria, especially morphological ones, for distinguishing the biotypes and for monitoring biotype development in the field. Liquido (1978), using a multivariate analysis with 11 morphological characters, could not discriminate between the biotypes. He also found no assortative mating or differences in chromosomes between them. Sogawa (1978b) found no significant differences among the biotypes in dimensions of the head capsule, hind tibia, tegmen, ovipositor or genital characters, but did find a statistically significant difference in the distribution of the spines on the hind tarsus. Sogawa (1978a) also suggested a difference in the frequency of certain esterase phenotypes between the biotype populations. However, neither of these differences were completely diagnostic. Recently Saxena & Rueda (1982) published detailed morphometric analyses using 109 different characters, from which they claimed reliable and complete separation of the IRRI biotypes. Their study deserves close examination as the results are contrary to previously published work.

The only other difference between the biotypes which has been frequently claimed is in fecundity (IRRI, 1977; Saxena & Rueda, 1982; Sogawa, 1981a). Biotype 3 has usually been reported as markedly less fecund than biotypes 1 or 2, even when reared on susceptible varieties.

Here we report the results of two sets of experiments designed to examine the above claims of differences in fecundity and of morphometric difference between the IRRI biotypes. It must be stressed that although we use the term biotype throughout, it is used merely as a label to identify the IRRI culture populations as the same as those used by previous authors. It does not signify an acceptance of them as discrete and separate categories and still less their applicability to other populations of *N. lugens* in different geographical regions.

Materials and methods

The insects used were subcultures of the biotype cultures maintained at IRRI. As at IRRI, biotype 1 was reared on TN1, biotype 2 on Mudgo and biotype 3 on ASD7. The cultures were maintained at Cardiff in screened cages in a glasshouse at 25 ± 2 °C.

Morphometric analysis. For the morphometric studies, ten characters were scored for 30 brachypterous ♂♂ of each of the biotypes. The characters used (Fig. 1) were selected on a basis of being easy to measure and giving reliable, consistent results. Attempts to repeat some of the measurements made by Saxena & Reuda (1982) were abandoned, as many were difficult and gave unreliable results.

For genitalia studies specimens were cleared in 10% KOH solution, dissected and mounted in glycerol on glass slides. Dry mounted specimens were used for body measurements. All measurements were made by tracing from camera lucida projections using a Lietz ASM Image Analyser. Counts of the numbers of teeth on the tibial spur and spines on the aedeagus were made under low power of a compound microscope.

The resulting data were analysed by a Wilks' Lambda method of discriminant analysis, as described by Klecka (1975). Two sets of experiments were done: (1) each biotype was reared on its normal host variety, and (2) each biotype was reared on the same susceptible variety (TN1). To achieve the latter, gravid females were removed from the culture and placed on TN1 plants. The resulting progeny were reared to adults and then the sample was taken for measurement.

Fecundity. Newly emerged brachypterous ♀♀ were collected, weighed and each placed with a ♂ in a 20 × 2.5 cm glass tube containing a rice seedling. After 72 h they were transferred to new seedlings. The seedlings were changed daily for the next seven days and the eggs laid in each one were counted. Again two sets of experiments were done: (1) each biotype was reared on its normal host variety and

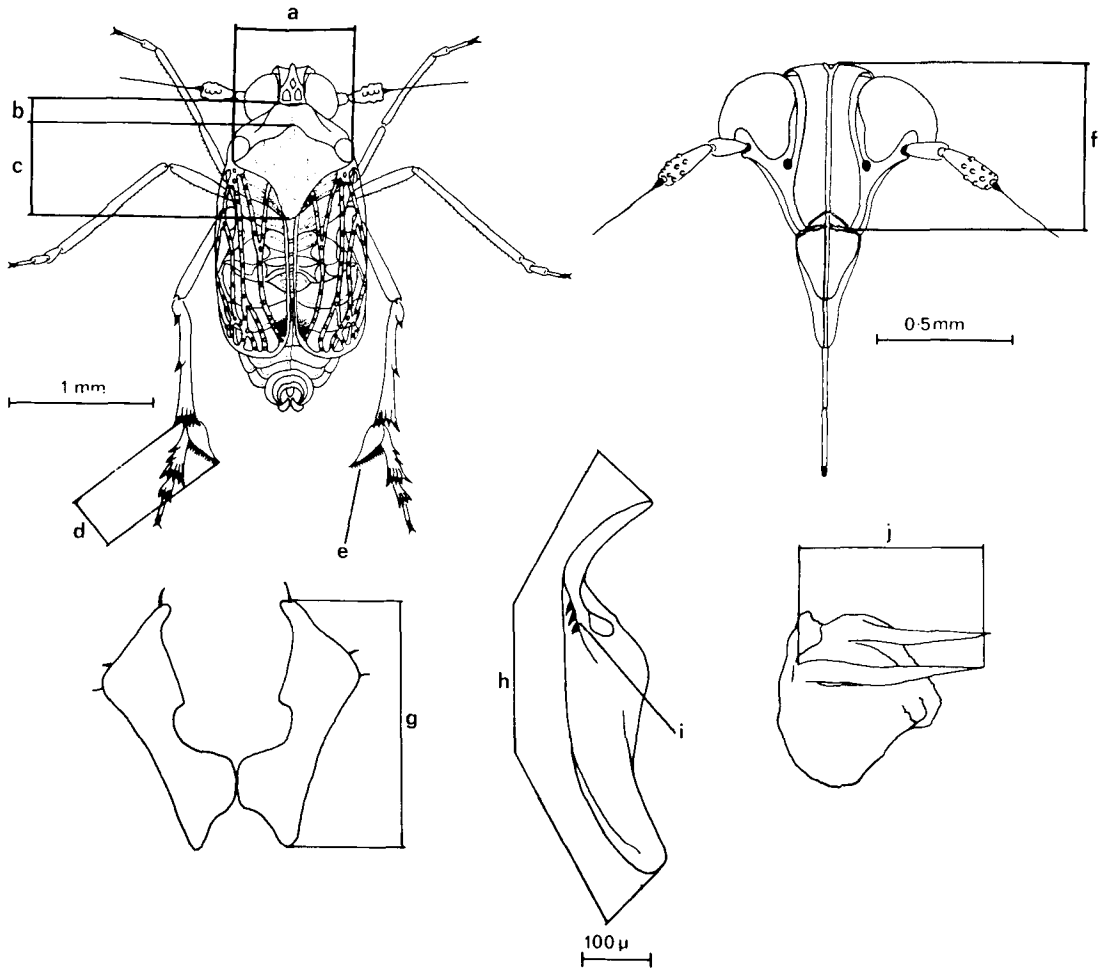

Fig. 1. Characters used in morphometric analysis: (a) scutellum width; (b) pronotum length; (c) scutellum length; (d) length of tarsal spur; (e) number of teeth on tarsal spur; (f) frons length; (g) paramere length; (h) aedeagus length along perimeter; (i) aedeagus spine number; (j) anal tube appendage length.

its fecundity tested on seedlings of the same variety, and (2) each biotype was reared on the susceptible TN1 and its fecundity tested on TN1 seedlings. Adults for this were obtained in the same way as those for the morphometric analysis.

During the experiments some females died, probably as a result of handling. Fecundity was determined by counting eggs laid for each day while each ♀ was alive and expressed as eggs per ♀ per day.

Results

Morphometric analysis. The results of our morphometric analysis are presented as scatter plots on a graph defined by the first two discriminant functions (Fig. 2). For the first set of experiments where each biotype was reared on its normal host variety, the plot reveals that biotype 1 forms a separate discrete grouping, but biotypes 2 and 3, although they differ significantly ($p < 0.05$) overlap widely (Fig. 2A). Predicted group membership based on these results gives for biotype 1, 100% correct assignment; for biotype 2, 67% correct, 3% as biotype 1 and 30% as biotype 3; and for biotype 3, 80% correct and 20% as biotype 2. Overall, 82% of individuals were correctly assigned to their known biotype. However, when all the biotypes were reared on TN1, a different picture emerges. The 3 groups coalesce and overlap extensively (Fig. 2B). They are not clearly separated even though the discrimina-

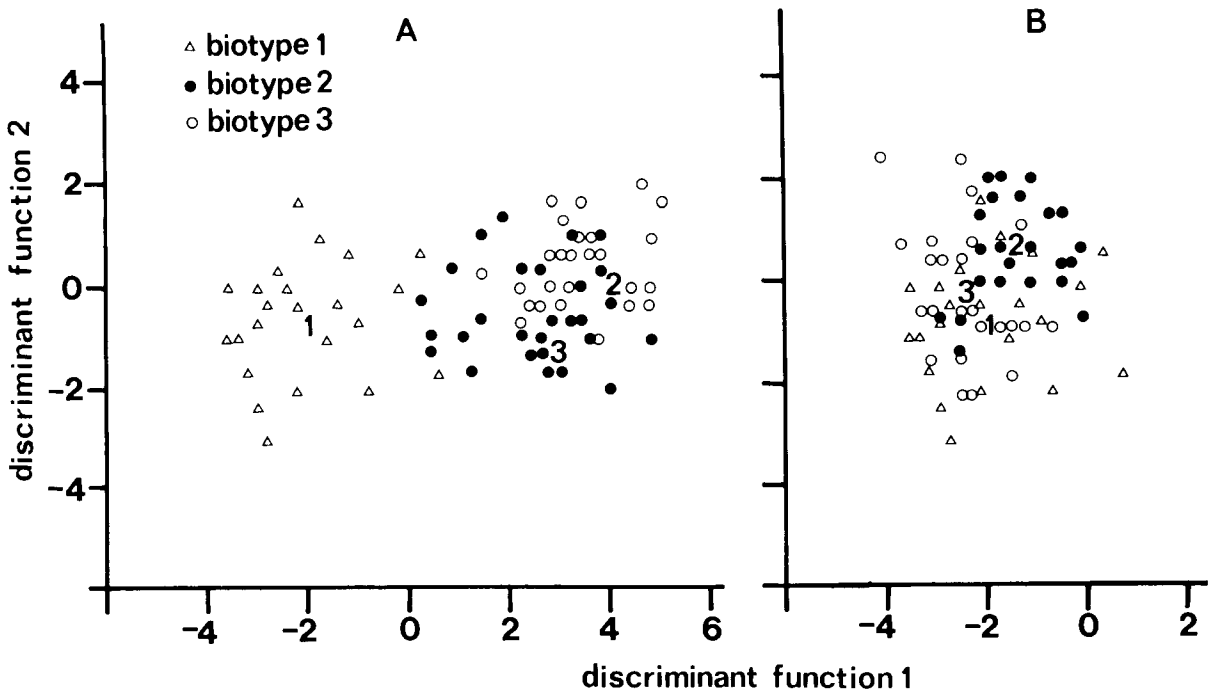

Fig. 2. Plots of discriminant function 1 against discriminant function 2 for each biotype of *N. lugens* on, (A) normal host and (B) TN1. Group centroids for each biotype shown by numbers corresponding to biotype.

tion is statistically significant ($p < 0.05$). For biotype 1 only 43% of individuals were correctly assigned with 30% classified as biotype 2 and 27% as biotype 3; for biotype 2, 60% were correctly assigned, 27% were classified as biotype 1 and 13% as biotype 3; and for biotype 3, 77% were correctly assigned and 13% were classified as biotype 2. The classification routine was only able to assign 60% of the individuals to the group from which they came.

The differences between the biotypes on their normal rice varieties were probably due to differences in the nutritional value of the three rice varieties. Individuals reared on TN1 were generally somewhat larger than those on resistant varieties. This is shown when each individual character is plotted for the biotypes both on their normal host and on TN1 (Fig. 3). For biotypes 2 and 3 all size related characters increased when they were reared on TN1. The aedeagus spine and tibial spur tooth count however remained essentially the same.

Fecundity. The number of eggs per ♀ per day of each biotype, when reared and tested on their normal host varieties were: 38.4 ($n = 26$, s.d. = 14.2) for biotype 1, 22.8 ($n = 43$, s.d. = 12.2) for biotype 2, and 24.9 ($n = 36$, s.d. = 11.9) for biotype 3. Biotypes 2 and 3 did not differ significantly, but both were significantly less fecund than biotype 1 ($p < 0.05$).

When biotypes 2 and 3 were both reared on TN1, fecundities improved markedly. Biotype 2 increased to 41.3 ($n = 43$, s.d. = 17.8) and biotype 3 to 29.8 ($n = 35$, s.d. = 12.7). Biotypes 1 and 2 were not significantly different from each other, but biotype 3 was significantly lower than both ($p < 0.05$). This lower fecundity of biotype 3 on TN1 corresponds to its slightly reduced body weight on TN1 compared to biotypes 1 and 2 (Table 1).

Table 1. Mean and standard deviations for weights of individual females from each biotype on normal host variety and on TN1.

	mean		
	n	wt	SD
Biotype 1 on TN1	27	2.46	0.55
Biotype 2 on Mudgo	45	2.33	0.44
Biotype 3 on ASD7	39	2.16	0.27
Biotype 2 on TN1	48	2.42	0.40
Biotype 3 on TN1	42	2.31	0.31

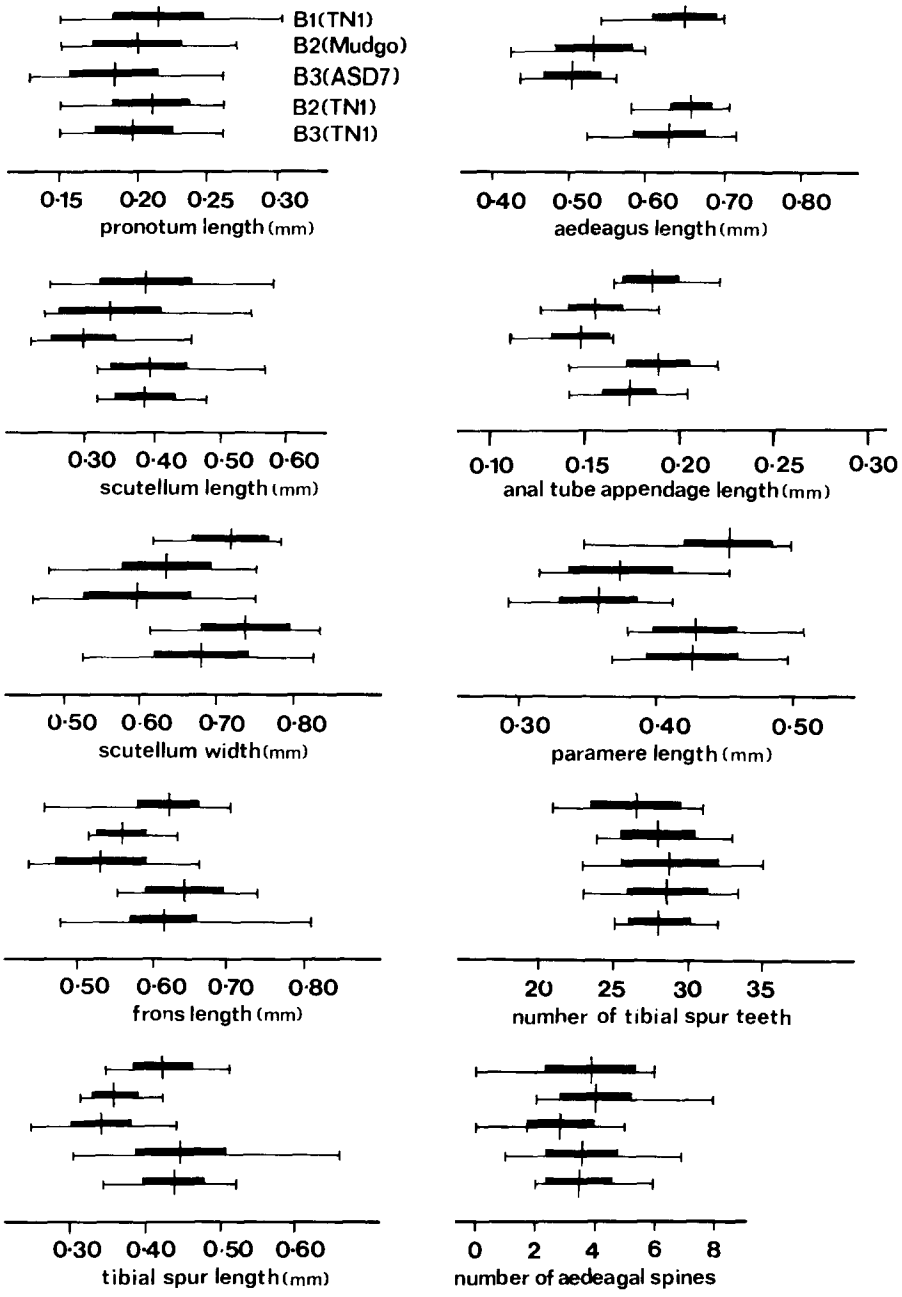

Fig. 3. Distribution of each character used in morphometric analysis of *N. lugens* in sequence: biotype 1 (B1) on TN1; biotype 2 (B2) on Mudgo; biotype 3 (B3) on ASD7; biotype 2 on TN1; biotype 3 on TN1. Each bar shows mean, SD and total range.

Discussion

The major differences between the biotypes of *N. lugens* consist of distinctive patterns of host virulence revealed by mass screening on test varieties. Until recently no other diagnostic differences between the populations had been positively demonstrated.

Saxena & Rueda (1982), in a very extensive analysis, have demonstrated significant morphometric differences between all three IRRI biotypes and claimed that they were able to achieve 100% discrimination between them. Some details of their results are puzzling. For example, it seems odd that almost totally different suites of characters provide the best discrimination between different wing morphs and sexes of the biotypes. Often characters from only one side (right or left) of the insect provided significant discriminators, while the same characters on the other side did not. Also the final table of their paper in which they show the percentage of successful biotype classification for individual insects does not give either the characters used or the equations for the boundaries between the biotypes. Saxena & Rueda suggested that their work might be used for identifying potential resistance breaking biotypes in the field, but this ommission makes their analysis difficult or impossible to use in practice.

Our own less extensive studies on only one morph and using fewer characters of the same three biotype populations on their normal hosts confirmed differences between biotype 1 and biotypes 2 and 3, but failed to distinguish completely between the latter. However, we certainly accept that it may be possible to distinguish biotypes 2 and 3 by using a more extensive array of characters, such as that used by Saxena & Rueda.

In order to establish the existence of a genetic basis for morphometric differences between the biotypes, it is necessary first to rear them all under the same conditions on the same host variety. In practice this can only be done on a susceptible variety. The results of our experiments show that differences between the biotypes are largely lost after only one generation on a common variety. This suggests that the differences between the biotypes are to a great extent environmentally induced and may be a result of nutritional differences between the cultivars, TN1, Mudgo and ASD7. Saxena & Rueda presented no comparable data to support their contention that the morphometric differences they described demonstrate genetic differentiation of the populations. Without such data, their claim cannot be upheld.

It is possible that some genetically determined morphometric differences between the IRRI biotypes may exist, but as yet they have not been unequivocally established. These biotype populations are long inbred laboratory cultures originally established 10 years ago and continuously reared on the same hosts. Such small inbred populations might be expected to have developed some genetic differences, either by differential selection or by founder effects in the small populations from which they were originally selected. If such differences have any significance outside the inbred IRRI cultures, it will be necessary to establish repeatable associations between virulence and morphometric characteristics. No such associations have been demonstrated to date.

Another major biological difference between the biotypes which has been claimed by different workers concerns the possible lower fecundity of biotype 3 when compared to biotypes 1 and 2 (IRRI, 1977; Sogawa, 1981a; Saxena & Rueda, 1982). Sogawa (1981a) stated that the number of nymphs produced per female and the population growth of biotype 3 was of the order of half that of biotypes 1 and 2 even on susceptible varieties. However, in Sogawa's study this was mainly due to less than half of the biotype 3 pairs being fertile. We suggest that such pairs probably were not mated and thus may have falsified estimates of fecundity. Also results at IRRI (IRRI, 1981, p. 56, and Fig. 3; IRRI, 1983, p. 62 and Fig. 2) indicate that population build-up of biotype 3 is very similar to that of the other two biotypes. Thus the published data are equivocal. In our experiments reported here, we did obtain a lower fecundity on TN1 for biotype 3 than for either biotypes 1 or 2, but certainly not as low as a half. Considering the apparent contradiction in the different published results, claims of a lower fecundity for biotype 3 need further substantiation.

The 'biotypes' of *N. lugens* differ in the patterns of virulence which they show to standard test cultivars in mass screening trials. The patterns may be independently acquired by different populations. The evidence for morphological or other biological criteria, which may be used to separate the 'bio-

types' has yet to be demonstrated conclusively. Even less have such differences been shown to be reliable field discriminators of virulence in different regions. Indeed the evidence suggests that virulence patterns have evolved independently many times and in different regions (Claridge & Den Hollander, 1982; Claridge *et al.*, 1982). Equally there is no justification for regarding the so called biotypes as preliminary stages in the process of speciation.

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Résumé

La signification des différences morphométriques et de fécondité entre des 'biotypes' de Nilaparvata lugens

Ce que l'on a appelé des 'biotypes' de *N. lugens* sont des populations caractérisées par différents types de virulence à l'égard de différents cultivars résistants de riz, mis en évidence par des essais variétaux systématiques. Différents chercheurs ont tenté de trouver des caractères morphologiques pour identifier ces biotypes.

Nous avons fait une analyse morphométrique des biotypes 1, 2 et 3 de l'International Rice Research Institute (IRRI) Philippines. Quand ils sont élevés sur leur propre varieté - biotype 1 sur TN1, 2 sur Mudgo, 3 sur ASD7 - des différences significatives sont observées, bien qu'il y ait un chevauchement considérable. Quand les 3 populations 'biotypes' sont élevées sur la variété sensible TN1, les différences morphométriques sont réduites et le chevauchement fortement augmenté. Nous pourrions alors conclure qu'une part importante de la différenciation morphométrique est due à des facteurs écologiques et non à des différences génétiques entre les populations. Des chercheurs avaient indiqué des différences de fécondité entre les biotypes de l'IRRI, le biotype 3 étant significativement moins fécond; les résultats publiés son contradictoires. Nos observations suggèrent une certaine diminution de la fécondité pour le biotype 3 élevé sur TN1, mais plus limitée que les autres auteurs ne l'avaient envisagée.

Nous en concluons qu'il n'y a pas de véritable preuve pour étayer l'hypothèse que les 'biotypes' de *N. lugens* sont caractérisés par des paramètres morphométriques génétiquement déterminés. Il est alors fallacieux de suggérer que de tels caractères pourraient être utilisés pour identifier des populations avec différents types de virulence. Nous repoussons aussi l'hypothèse que les 'biotypes' représentent une étape dans le processus de spéciation.

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