Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 96, No. 4, July 1987, pp. 425–435.

Nature of resistance in selected rice varieties and population fluctuation of green leafhoppers, *Nephotettix virescens* (Distant) and *Nephotettix nigropictus* (Stål)

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Abstract. In multiple choice tests with 30-day-old plants of resistant and susceptible varieties, both the species of green leafhoppers, *Nephotettix virescens* (Distant) and *Nephotettix nigropictus* (Stål) exhibited relative non-preference to highly resistant varieties (Ptb 2, Ptb 7 and Ptb 18) both for settling and oviposition. Even when 100 first instar nymphs were caged on individual 25-day-old plants of highly resistant varieties, the plants suffered very low damage and also induced high mortality of nymphs. Different ages of the plants had no influence on the antibiosis mechanism of resistant varieties. Feeding behaviour studies revealed that both the species made more punctures and excreted less honeydew while feeding on resistant varieties than on susceptible ones. Histological studies indicated no mechanical barrier for feeding in resistant varieties.

Field investigations indicated that resistant varieties viz Ptb 18, Ptb 2 and Ptb 7 harboured less population of green leafhoppers and had relatively nil or very low incidence of tungro virus disease. An year round survey in the rice ecosystem and marshy habitat where *Leersia hexandra* grows in abundance revealed that although both the species coexisted in rice fields, *Nephotettix nigropictus* alone was present in the marshy habitat.

Keywords. *Nephotettix virescens*; *Nephotettix nigropictus*; nature of resistance; population fluctuation.

1. Introduction

The current trend in agricultural pest control programmes in various rice growing countries is towards evolving a sound pest management system. It requires a proper understanding and implementation of the population regulating factors such as cultural control including growing resistant varieties. In India, screening for resistance to green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stål), was initiated at the national headquarters of the All India Coordinated Rice Improvement Project (AICRIP 1969) and also at the Central Rice Research Institute (CRRI 1970).

In view of the increased importance of green leafhoppers in various states of the country, we investigated the insect-host interaction at the Directorate of Rice Research, Hyderabad (formerly AICRIP). Earlier, Viswanathan and Kalode (1984) reported 10 rice varieties to be greatly detrimental to insect bionomics using 1st instar nymphs and adults of both green leafhoppers (*N. virescens* and *N. nigropictus*). We now describe the mechanism of resistance in selected rice varieties and population fluctuation in rice ecosystem.

2. Materials and methods

2.1 *Preference/non-preference mechanism*

2.1a *Settling and ovipositional response to susceptible TN 1 rice plants of different ages*: TN 1 plants of different ages were grown randomly in a wooden tray. Plants of the same ages were replicated 5 times. Gravid females were released in a cage containing the tray. Observations on settling preference and egg laying were made by counting the insects and dissecting plants for recording number of eggs.

2.1b *Preference/non-preference response of adults to selected rice varieties*: Eight plants of each variety (Ptb 18, Ptb 2, Ptb 7 and TN 1) were grown randomly in 3 replications in wooden trays at a spacing of 10 × 10 cm and only the mother tiller was allowed to grow. When plants were 30-day–old, a total of 320 insects in a sex ratio of 1 : 1 were released and observations were taken on the insect settling pattern at every hour upto 7 h and at 24, 30, 48 and 54 h.

2.2 *Studies on antibiosis*

2.2a *Effect of higher population on antibiosis reaction*: The high antibiosis reaction exhibited in the selected resistant varieties (Ptb 18, Ptb 2 and Ptb 7) was further confirmed by caging 100 first instar nymphs per 25-day–old plants. Seven replications were maintained for each test variety. Damage caused was assessed at 5, 10, 15 and 20 days after insect release and the damage percentage was calculated as follows

$$\text{Damage (\%)} = \frac{\text{No. of leaves damaged}}{\text{Total No. of leaves}} \times 100$$

2.2b *Effect of plant age on antibiosis*: To study the influence of plant age on antibiosis, known number of first instar nymphs were caged on 10, 25, 45 and 90-day–old plants. Three replications were maintained for each age group of varieties. Surviving nymphs were counted daily until 7 days.

2.3 *Population buildup on selected varieties*

2.3a *Greenhouse test*: The population buildup was studied by releasing 5-day–old 5 females and males on 25-day–old plants of test varieties. Each variety was replicated 10 times, population buildup was recorded 35 days after infestation.

2.3b *Field test*: To determine population fluctuation of the two green leafhopper species and the incidence of rice tungro virus, a field trial was conducted at the AICRIP experimental farm during *kharif* season. In addition to the 3 highly resistant varieties, one moderately resistant variety (IR 8) and one susceptible variety (TN 1) were also included. The selected varieties were planted in a randomized block design and the plot size was 16 sqm. Each variety was replicated thrice. Bordering the test varieties, the susceptible TN 1 was planted horizontally so as to increase the insect population. Ten days after transplanting, 50 oviposited pots from greenhouse were kept on both sides of the plots along TN 1 lines to increase the insect pressure in the experimental field. Observations were made on 30 hills of each variety at every 10 days upto 80 days after transplanting. Tungro virus incidence was recorded at 60 days after transplanting based on disease symptomology.

2.3c *Population fluctuation*: Using a sweepnet, the two species of green leafhoppers were sampled periodically in rice fields, rice fallows and surrounding swampy areas to obtain supplementary information on natural population fluctuations.

2.4 *Feeding behaviour on selected varieties*

2.4a *Honeydew excretion*: In greenhouse experiments, both third instar nymphs and adults were kept without food for 2 h and caged separately on single leaf in special glass cages. Each variety was replicated 5 times. Two male and female insects each were caged when adults were used for test. Similar tests were carried out by caging 4 third instar nymphs. Honeydew droplets were collected on Whatman filter paper by adopting technique similar to that described by Reddy and Kalode (1985). After 24 h, the insects were removed and the extent of honeydew excreted was visually assessed by spraying the Whatman filter paper with ninhydrin reagent (0·02%), which reacts as pinkish yellow spots with the amino acids present (IRRI 1968).

2.4b *Probing marks*: The number of feeding marks made on the test variety was determined by caging a single insect for 6 h by restricting it on a single leaf of 25-day–old plant. There were 5 replications. Then the leaf was dipped in 1% erythrosin solution which stained the feeding marks as per the method suggested by Naito (1964).

3. Results and discussion

3.1 *Preference/non-preference mechanism*

Greenhouse investigations in relation to plants of different ages of the susceptible variety TN 1 showed that *N. virescens* preferred 30-day–old plant, while *N. nigropictus* preferred 40-day–old plant both for settling and egg laying (figure 1). Multiple choice studies using resistant and susceptible varieties indicated that highly resistant varieties were relatively less preferred by both the species for settling as well as egg laying. The insects did alight on the resistant varieties (figure 2), but only by about 7 h after their release, majority of them were found to locate the suitable host. Thus, selection of a suitable host by these species appeared to be a passive one. In similar studies Cheng and Pathak (1972) noted that the green leafhopper (*N. virescens*) differed in their preference for various varieties. In spite of adult-non-preference for different varieties, several of the resistant varieties received as many eggs as the susceptible variety. Likewise Choi *et al* (1979) reported for brown planthopper that resistant varieties which were non-preferred for feeding did not exhibit the same trend towards oviposition. The preference response of the insect to a variety is more pronounced after 48 h of the infestation (IRRI 1977). Such response is presumed to be caused by the lack of certain stimuli or by the presence of a strong repellent in resistant varieties.

3.2 *Studies on antibiosis mechanism*

3.2a *Effect of higher insect population on antibiosis*: The detailed studies with selected resistant varieties (Ptb 2, Ptb 7 and Ptb 18) revealed a high degree of anti-

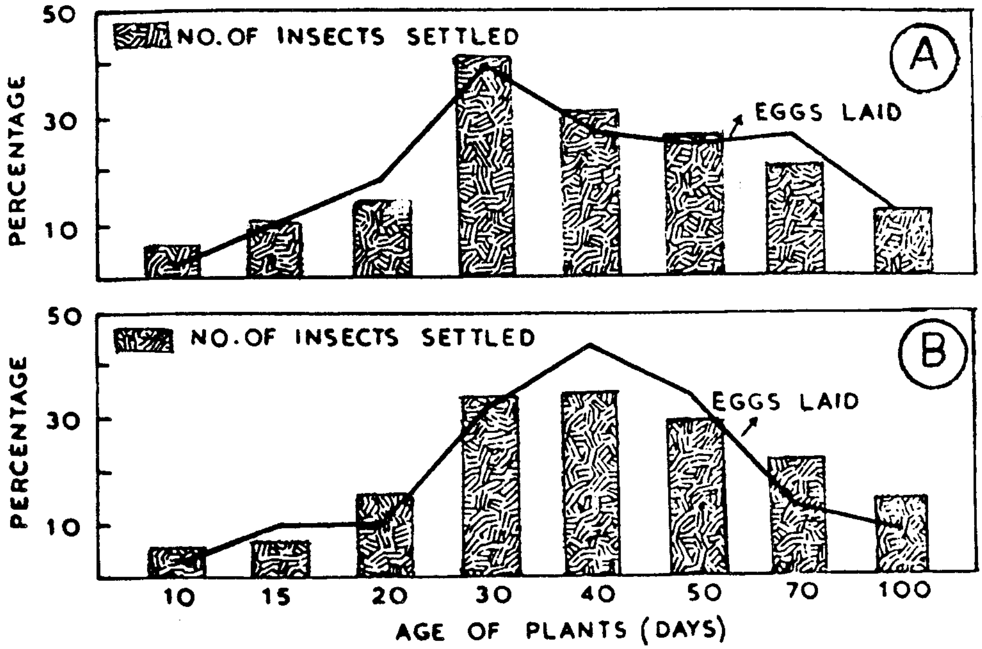

Figure 1. Preferential response for settling and oviposition by *N. virescens* (A) and *N. nigropictus* (B) for plants of different ages of the susceptible rice variety TN 1.

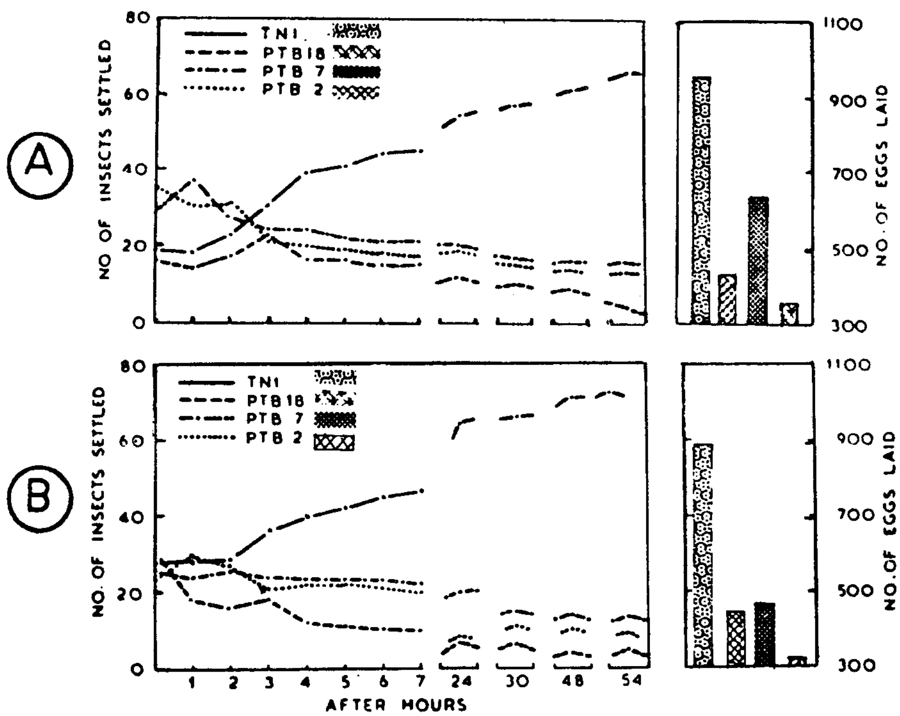

Figure 2. Preferential response for settling and oviposition by *N. virescens* (A) and *N. nigropictus* (B) for different resistant and susceptible rice varieties.

biosis to both *N. virescens* and *N. nigropictus* (Viswanathan and Kalode 1984). Even with release of 100 first instar nymphs per plant, in the present studies, the damage on resistant varieties was considerably low (figure 3) since there was a high mortality of insects and only meagre population could survive on these varieties. Among the highly resistant varieties, Ptb 18 and Ptb 2 proved to be relatively better than Ptb 7 for both the species. It was evident that resistant varieties could drastically reduce the insect population caged on them. This population reduction could primarily be attributed due to their high antibiosis. Such type of population reduction in *N. virescens* and *Nilaparvata lugens* (Stål) due to antibiosis mechanism is known in rice varieties (Cheng and Pathak 1972; Sogawa 1973; Karim 1975; Karim and Pathak 1978; Heinrichs and Rapusas 1984). High rate of initial nymphal mortality on resistant varieties has been noted for *N. cincticeps* (Kawabe 1985). The present results also reveal this trend for both *N. virescens* and *N. nigropictus*. However, high level of initial nymphal mortality was not observed in case of brown planthopper as only

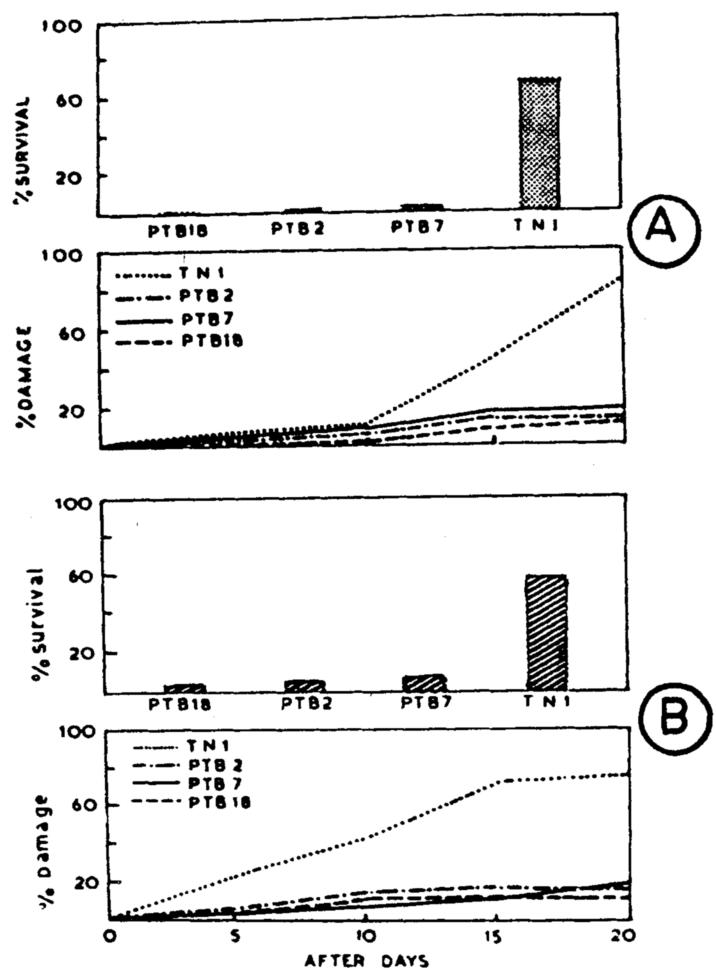

Figure 3. Plant damage (%) and survival of *N. virescens* (**A**) and *N. nigropictus* (**B**) on different rice varieties when caged with 100 first instar nymphs.

19·5–30% nymphal mortality was recorded upto 6th day of caging on resistant varieties (Reddy and Kalode 1985).

3.2b *Effect of plant age on antibiosis*: Age of the plant showed no significant influence on antibiosis mechanism in highly resistant varieties studied. On the susceptible TN 1, higher nymphal population (88–96·5%) of both the species survived for 7 days after release on plants of different age groups. But on resistant Ptb 18, within 5 to 6 days, there were no surviving insects (*N. virescens*) on 10, 25, 45 and 90 day old plants. Similarly, plants of different ages of other resistant varieties (Ptb 2 and Ptb 7) did not show any significant differences in relation to survival of nymphs. No surviving *N. nigropictus* nymphs were observed beyond 4 days on Ptb 18 plants of 90 day old while on other ages of the same variety, a low population was recorded. At the end of 7 days, either there were no surviving insects or meagre population on plants of different ages of Ptb 2 and Ptb 7. The observations made at the International Rice Research Institute (IRRI 1968) support present findings where the age of the varieties did not alter the resistance of rice varieties to green leafhoppers and brown planthoppers. In contrast, some amount of fluctuation in levels of antibiosis with the growing stages of the resistant rice varieties has been reported for *N. cincticeps* (Kishino and Ando 1979). Cartier (1963) noted that absolute resistance of peas to *Acyrthosiphon pisum* (Harr.) increased with age of the plants, but relative varietal differences remained constant during most of the life of the host plant.

3.3 *Population buildup on resistant and susceptible varieties*

3.3a *Greenhouse test*: The population increase from 5 pairs at 35 days of release was 297 (115 adults) in case of *N. virescens* and 123 (69 adults) in case of *N. nigropictus* on susceptible TN 1 as compared to 7 (*N. virescens*) and 11·5 (*N. nigropictus*) per plant of Ptb 7. On other resistant varieties both the species did not survive except 1·5 insects (*N. nigropictus*)/plant of Ptb 2.

3.3b *Field test*: High level of resistance in Ptb 18, Ptb 2 and Ptb 7 as observed in the greenhouse was further confirmed in field experimentation (figure 4). Both the species indicated close similarity in population buildup showing peaks at 60 days after transplantation though the number of *N. nigropictus* was relatively less than that of *N. virescens* particularly on susceptible variety TN 1 and moderately resistant IR 8. Thus highly resistant varieties could suppress the field population of the green leafhoppers. Similar observations were made by Jayaraj (1967a) who noted that *Empoasca flavescens* (F) populations were more on susceptible and tolerant castor varieties than on resistant varieties. Root and Olson (1969) also found that host species and varieties had a definite influence on rate of population development of the aphid, *Bravicoryne brassicae* (Lin.).

It is interesting to note that no tungro virus disease symptoms were manifested on varieties Ptb 18 and Ptb 2, while it was considerably low (4·4%) on Ptb 7 (table 1). Probably, the above varieties did not suffer by this disease because of their high resistance and consequent less insect population. This again upholds the view that the use of varieties highly resistant to green leafhoppers could possibly check tungro spread.

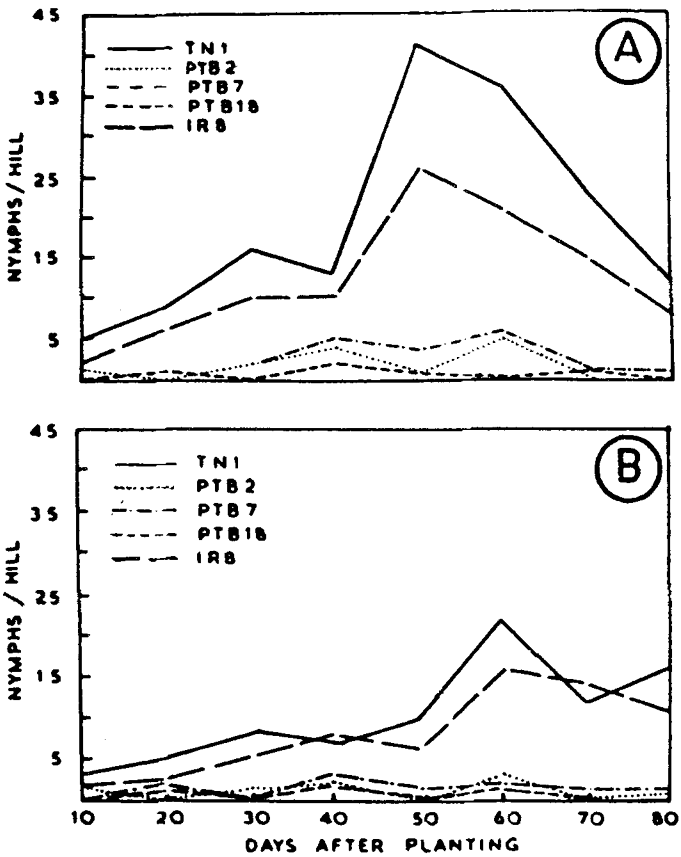

Figure 4. Population fluctuation of *N. virescens* (A) and *N. nigropictus* (B) on different rice varieties in the field experiment.

Table 1. Percentage of plants infected with tungro disease in field studies with selected rice varieties.

Variety	Damage rating	Plants infected with tungro (%)*
Ptb 2	Resistant	0
Ptb 7	"	4
Ptb 18	"	0
TN 1	Susceptible	97
IR 8	Moderately resistant	90

*Average of 3 replications.

3.3c *Pattern of population fluctuation in rice fields in marshy habitat*: Observations made in rice fields (figure 5) revealed that during August to September, both the species were comparatively meagre but started building up from September onwards. *N. virescens* exhibited 3 peaks from November to January. Similar increase of *N. nigropictus* was observed, except during November. However, the population of

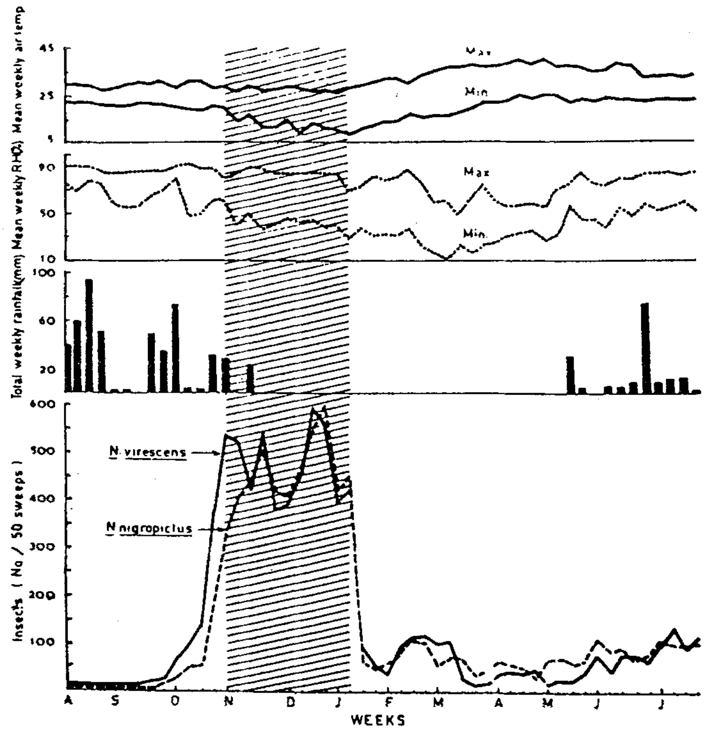

Figure 5. Population fluctuation of *N. virescens* and *N. nigropictus* adults in rice fields.

both the species declined from January 2nd week onwards upto 4th week of July recording low population. During peak periods the maximum temperature and relative humidity were 29–39°C and 80–90%, respectively.

Survey of the marshy habitat showed that *N. virescens* was completely absent throughout. On the other hand, *N. nigropictus* was found building up from October onwards with a peak in March which was in far greater number in comparison to rice fields. However, there was a fall in population from second week of April in this habitat. The main host plant species in this situation was *Leersia hexandra*. *L. hexandra* was found to be the most suitable host for *N. nigropictus* (Viswanathan and Kalode 1986).

3.4 *Feeding behaviour*

3.4a *Amount of feeding—honeydew excretion*: Studies on the honeydew excretion of the two species feeding on resistant and susceptible varieties revealed that both the species fed less and excreted less on highly resistant varieties (table 2). Comparable observations have been made with resistant varieties by Cheng and Pathak (1972) with green leafhopper and in case of brown planthopper by Sogawa (1973), Kalode and Krishna (1979) and Reddy and Kalode (1985). In contrast, the honeydew excreted by *N. cincticeps* on resistant rice plants was by far more in quantity than those on susceptible plants (Kawabe 1985). Recent studies at IRRI have shown that though

Table 2. Relative amount of honeydew excreted on selected rice varieties by green leafhoppers.

	Relative amount of honeydew excreted			
	N. virescens		*N. nigropictus*	
Variety	Nymph	Adult	Nymph	Adult
Ptb 2 (R)	+	+	+ +	+
Ptb 7 (R)	+ +	+	+	+
Ptb 18 (R)	+ +	+	+	−
TN 1 (S)	+ + +	+ + +	+ + +	+ + +

+ + +, Large amount; + +, moderate amount; +, trace amount; −, nil; R, resistant; S, susceptible.

Table 3. Feeding punctures made by *N. virescens* and *N. nigropictus* adults on the leaf blades of selected varieties.

	Punctures/insect/h					
	N. virescens			*N. nigropictus*		
Variety	Min	Max	Mean	Min	Max	Mean
Ptb 18 (R)	6·1	12·5	9·2	5·8	13·2	10·1
Ptb 2 (R)	5·3	10·2	7·1	6·1	14·7	9·3
Ptb 7 (R)	4·8	9·6	6·6	5·2	10·4	7·1
TN 1 (S)	3·2	4·7	3·4	4·4	5·1	4·6

R, Resistant; S, susceptible.

the total area of honeydew spots were similar when green leafhopper fed on resistant or susceptible varieties, xylem feeding increased with the level of resistance (IRRI 1982).

3.4b *Amount of feeding—probing marks*: Both *N. virescens* and *N. nigropictus* were found to make more feeding punctures (6·6–10·1) on resistant varieties as compared to 3·4–4·6 on susceptible varieties (table 3). Similar observations have also been recorded in rice varieties to brown planthopper and green leafhopper (Sogawa and Pathak 1970; Cheng and Pathak 1972; Karim 1975; Reddy and Kalode 1985; Heinrichs *et al* 1985; Khan and Saxena 1985b). Histology of fed tissue showed that there were no physical or mechanical barriers to prevent the insect feeding on resistant varieties where majority of the stylet sheaths reached vascular bundles (table 4). Sogawa and Pathak (1970) also did not find any mechanical barrier to feeding by *N. lugens* in the resistant variety, Mudgo. Cheng and Pathak (1972) concluded that resistance to *N. virescens* cannot be attributed to mechanical factors. Similar conclusions were drawn against *N. cincticeps* by Oya (1980) and Oya and Saito (1981). However, Peraiah *et al* (1979) stated that brown planthopper resistant varieties ARC 6650 and Ptb 33 had thicker hypodermis and an extra sclerenchymatous band in the stems which offered mechanical resistance to the insect. Resistance to *E. flavescens* in castor varieties was attributed to the anomalous thickening around vascular bundles by Jayaraj (1967b).

Table 4. Nature of salivary sheaths produced by *N. virescens* and *N. nigropictus* on selected rice varieties.

Stylet sheath	Rice varieties							
	N. virescens				*N. nigropictus*			
	TN 1 (S)	Ptb 2 (R)	Ptb 7 (R)	Ptb 18 (R)	TN 1 (S)	Ptb 2 (R)	Ptb 7 (R)	Ptb 18 (R)
Total No. of salivary sheath observed	98	107	115	90	78	91	120	67
Percentage salivary sheaths with 2 branches	2	0	4	6	1	0	2	1
Termination of salivary sheath (%)								
Phloem	68	56	71	71	80	66	79	74
Xylem	12	25	22	13	12	10	15	14
Parenchyma	20	19	7	16	8	24	6	12

S, Susceptible; R, resistant.

Thus, the above observations made on the feeding behaviour of *N. virescens* and *N. nigropictus* suggest that the resistance should primarily be due to certain biochemical factors which adversely affect the normal feeding sequence on resistant varieties. This is supported by Khan and Saxena (1985b) who showed that *N. virescens* fed primarily from the phloem of susceptible variety TN 1 but switched to the xylem on resistant varieties. In addition, the insects feeding behaviour on TN 1 plants could be disrupted by spraying the plant by steam distillate extract of resistant ASD 7 (Khan and Saxena 1985a). Further, it is evident that resistant varieties can be effectively used to suppress pest buildup in pest management programmes.

Acknowledgements

We thank Drs S V S Sastry and W H Freeman for providing facilities.

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