

**INFLUENCE OF SURFACE LIPIDS OF SOME RICE
(*Oryza sativa* L.) VARIETIES ON THE FEEDING
BEHAVIOR OF *Nilaparvata lugens* Stal,
(HOMOPTERA: DELPHACIDAE)**

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ABSTARCT

The rice brown plant hopper (BPH), *Nilaparvata lugens*, is a phloem-feeder on rice. Experiments were conducted to determine the influence of the surface lipids of two resistant (R) varieties, Ptb 33 and ARC 6650, two moderately resistant (MR) varieties Bg 379-2 and Bg 300 and two susceptible (S) varieties Bg 380 and TN1 on the feeding behavior of the insect. Ptb 33 had more surface lipids (0.25% fresh weight) followed by ARC 6650 (0.2%), Bg 300 (0.16%), Bg 379-3 (0.1%), Bg 380 (0.07%), and TN1 (0.08%). On S varieties the insect spent an average of 8 minutes for one feeding bout and moved 4 cm in 2 movements during a 30-minute period. On Ptb 33 and ARC 6650 it took an average of 4 minutes for a feeding bout and moved 6.5 cm in 5 movements. The insect moved more and spent less for feeding on para film wrapped plants than on normal plants. On surface-washed (dewaxed) Ptb 33, ARC 6650 and Bg 300 the insect moved less and spent more time for feeding than they did on unmodified plants, and did vice versa on Bg 380, TN1 and Bg 379-2. Brown planthoppers gained more weight and excreted more honeydew on de-waxed Ptb 33, ARC 6650 and Bg 300 as compared to normal plants and did vice versa on Bg 380, TN1 and Bg 379-2. It produced 13 times less honeydew and lost 18% weight on parafilm wrapped plants than on unmodified normal plants. These observations indicated that the surface lipids are responsible for BPH resistance in Ptb 33, ARC 6650 and Bg 300. The BPH resistance in Bg 379-2 could be due to factors other than surface lipids.

KEY WORDS: Brown planthopper, Rice, Surface lipids, Varietal resistance

INTRODUCTION

The rice brown planthopper, *Nilaparvata lugens*, is a phloem-feeder with a very high degree of host-specificity to feed and survive on rice, *Oryza* spp. (Wilson and Claridge, 1991). Resistance to *N. lugens* has been found in a wide range of traditional varieties of

cultivated rice (*O. sativa*) and in some wild *Oryza* spp. Some of these resistance has successfully been incorporated into varietal gene-bases in many Asian countries (Pathak and Khush, 1979 and Heinrichs *et. al.*, 1985) and has helped increase rice production and reduce costs related to insecticide application. The instability of the varietal resistance due to the rapid adaptation of the pest to previously resistant varieties requiring replacements with different resistant bases is a major constraint to the continued national rice improvement programmes in Asian countries. Therefore, efforts are being made to study the factors responsible for resistance in rice to this pest and help improve utilization of plant resistance in pest management (Cook *et. al.*, 1987 and Woodhead and Padgham, 1988).

Chapman (1977) indicated the importance of the plant surface in insect-plant-interactions and suggested it act as a defense against insect attack. It has been well established that phytophagous insects examine the plant surface and receive cues as to whether the plant is suitable for settling, feeding and oviposition (Woodhead and Chapman, 1986). Cook *et.al.*, (1987) and Woodhead and Padgham (1988) demonstrated the chemical composition of the surface wax of IR 46 -a rice variety moderately resistant to *N. lugens* - was responsible for the increased activity of the insect on that variety. They also suggested that the differences in *N. lugens* behaviour on different rice varieties could partly be due to the chemical composition of the surface waxes of those varieties, and unlikely due to the physical factors such as trichomes, surface silicification and morphology of surface lipids.

The labial tip of *N. lugens* is equipped with a battery of sensory receptors (Foster *et al.*, 1983a and b) and on resistant varieties it become restless (Woodhead and Padgham, 1988) and make more labial contacts with the plant surface without successful penetrations into the phloem (Nugaliyadde and Wilkins, unpublished). It is therefore hypothesized that the epicuticular waxes may contain factors that could confer resistance to the insect. In this study the influence of the surface lipids of six rice varieties, possessing different degrees of resistance to the pest, on the feeding and feeding related behavior of *N. lugens* is determined.

MATERIALS AND METHODS

Culture of test insects and plants

One-day-old *N. lugens* females from a population maintained on caged TN 1 plants in the greenhouse (25⁰ - 30⁰C, 60-80% r.h.) at the Rice Research and Development Institute (RRDI), Batalagoda, Ibbagamuwa was used in all experiments. The six rice varieties selected were; Ptb 33 and ARC 6650 (highly resistant- photoperiod sensitive traditional varieties), Bg 379-2 and Bg 300 (moderately resistant modern varieties with resistance derived from Ptb 33); Bg 380 and TN1 (highly susceptible modern varieties).

The test plants and the insect culture plants were grown on field plots at the experimental farm at RRDI. Chemical analysis was done at the Department of Agricultural and Environmental Science, University of Newcastle upon Tyne, NE1 7RU, United kingdom on plants grown in the glasshouse at Close House Field Station at 22⁰ -27⁰C, 70-90 r.h. and 12>h photoperiod. Eight-week old plants of the test varieties were used for the tests and for chemical extracts.

Influence of modified plant surfaces on insect activity and feeding

Main tillers, separated from uprooted hills, were used. In order to prevent the plant from desiccation the roots were immersed in water in a test tube clamped to a holder. A one-day-old brachypterous BPH female, starved for 2 h was placed just above the water level and observations were made on its activity for 30 minutes. Using the procedure described by Woodhead and Padgham (1988), the time spent at a point, and the distance, direction and time spent for each movement were recorded. These observations were made in the laboratory at 25-30⁰ C and 60-80% r.h. between 09:00 h to 15:00 h daily one plant at a time.

The above observations were also made on plants with the leaf-sheath covered by a single layer of stretched parafilm (Parafilm M: American National Can - stretched 2 times length and breadth wise) and on those where the leaf-sheath surface has been washed three times by chloroform (5 ml per plant). Thirty observations were made on each variety-treatment combination selected at random over a period of 10 weeks. Different insects were used for each observation.

Honeydew production and weight change

Newly emerged (one-day-old) brachypterous females were weighed individually and enclosed separately in parafilm sachets (5 cm x 5 cm) attached to the leaf sheath of the test plants planted individually in clay pots (15 cm dia.). The pots were then placed in a water-filled cement trough in the greenhouse at RRDI. After 24 h the weight of the insect and the excreted honeydew were recorded.

As in the pervious experiment, observations were also made on plants with the leaf-sheath covered by stretched para film and on plants where the leaf-sheath surface has been washed three times with chloroform (5 ml per plant). Using different plants and insects thirty observations were made for each variety-treatment combination. The experiment was conducted over a period of 10 days with three replications per treatment per day.

Extraction of surface lipids

The method describe by Woodhead and Padgham, (1988) was followed. Leaf-sheaths of 8-week-old plants were weighed and immersed separately in chloroform (approx. 20 ml per tiller) for 30 seconds. Using a graph paper with mm divisions the leaf-sheath area of each tiller was measured before immersing in the solvent. The extracts were filtered using nylon membranes (0.2 micron - Alltech Associates) and kept inside a fume cupboard until the solvent is completely evaporated. The resultant whitish wax-flakes were weighed and stored at - 20⁰C.

Fractionation of surface lipids

Thin layer chromatography (TLC) of the wax constituents was done on silica gel plates (Merck: Kieselgel 60 F₂₅₄, layer thickness 0.2 mm). The wax, dissolved in chloroform was applied on the plate as a band and the chromatogram was developed with chloroform: ethyl acetate: formic acid (50: 50: 0.5 v/v). After drying, the plate was viewed under UV light and the different fractions (bands) were marked. These bands were then scraped off the plate and extracted with chloroform. The isolated fractions were weighed and stored at -20⁰ C.

RESULTS AND DISCUSSION

Influence of modified plant surfaces on insect activity and feeding Behavioural observations:

On susceptible (S) varieties (TN1 and Bg 380), insects spent an average of 7-9 minutes for one feeding bout, moved an average of 4 cm in 2 movements, during the first 30 minutes (figure1). On resistant (R) varieties (ARC 6650 and Ptb 33) a feeding bout took an average of 3.9 minutes, and the insects moved an average of 6.5 cm in 5 movements.

In general, the insect made more number of movements and spent less time for feeding on parafilm wrapped plants than on normal plants. On dewaxed Ptb 33 the insects moved less and spent more time feeding than they did on normal plants, and did *vice versa* for dewaxed and normal susceptible and moderately resistant varieties. The number of movements made on dewaxed ARC 6650, Ptb33 and Bg 300 plants were significantly lower than those made on wrapped plants. The number of movements and the distance moved on wrapped and dewaxed susceptible (Bg 380 and TN1) plants did not differ much. The exception being on Bg 379-2 the insect moved more on dewaxed plants than on wrapped plants.

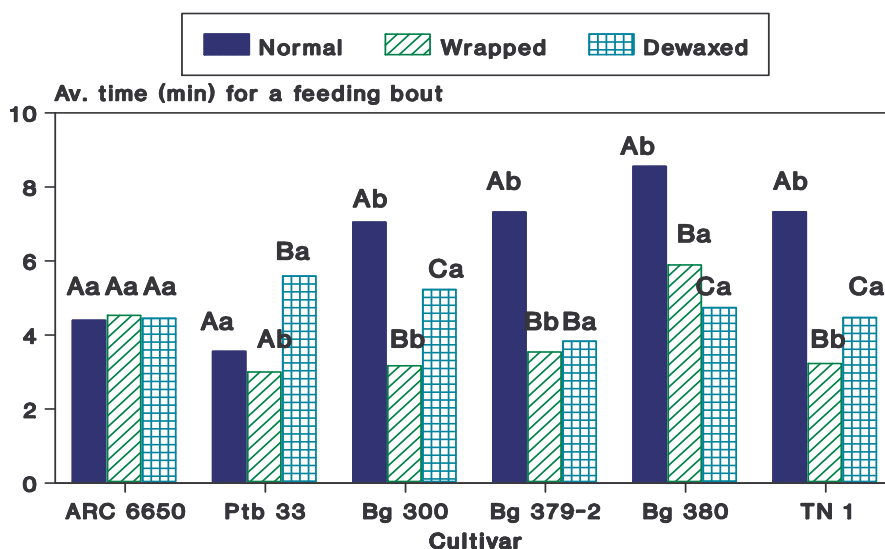


Figure 1. Time (min.) spent by *N. lugens* for a feeding bout on unmodified and modified rice-plants – (wrapped with parafilm and dewaxed with chloroform). (Upper and lower case letters indicate significance ($0.05 < p$) within a variety and within a treatment respectively)

Since there were linear relationships among the number of movements, the time spent for one feeding bout and the distance moved on each variety or variety/treatment combination the following formula was developed to estimate the relative suitability of a particular surface for the insect.

$$\text{Suitability Index (SI)} = \frac{\text{Av. time spent for a feeding bout} \times 100}{\text{No. of movements} \times \text{average distance moved}}$$

Bg 380 (S) and TN 1 (S) were the most suitable (SI >90) varieties followed in order by Bg 379-2 (MR), Bg 300 (MR), ARC 6650 (R) and Ptb 33 (R) (figure 2). The difference between susceptible (SI >90), moderately resistant (SI 50-60) and resistant groups (SI 10-20) were significant. Wrapped plants reduced the suitability irrespective of the varietal resistance. The reduction between unmodified (or normal) and wrapped plants were significant ($p < 0.05$). Dewaxed Ptb 33 (R) was superior to normal Ptb 33 plants, however, all other varieties reduced their fitness once the surface wax is removed. Among the dewaxed plants, Ptb 33 (R) and Bg 300 (MR) recorded significantly higher suitability than all other varieties. The

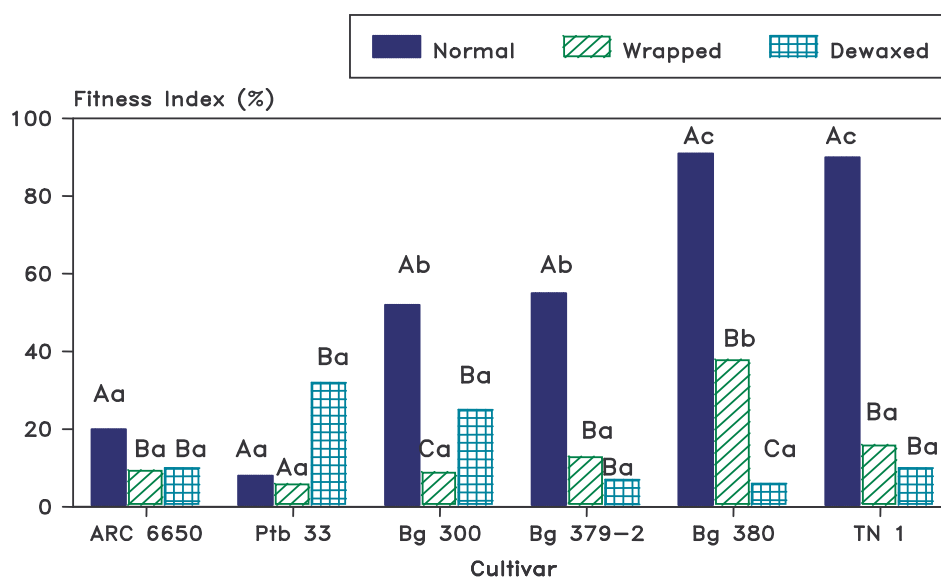


Figure 2. Estimated suitability indices for the unmodified and modified plant surface of rice varieties to the *N. lugens* (letters in upper and lower case letters indicate significance ($0.05 < p$) within a variety and within treatment respectively.)

differences between dewaxed plants and wrapped plants were not significant for ARC 6650 (R), Bg 379-2 (MR), and TN1 (S).

These behavioural observations indicated that the surface lipids of Ptb 33 contains compounds that could act as deterrents to *N. lugens*. The dewaxed Ptb 33 plants, which may have completely or partially lost the deterrent compounds, became more suitable for the insect. It is also possible that the remaining epicuticular wax, which has not been washed, act as an arrestant to the insect. The reactions of the insect to dewaxed susceptible plants gave further evidences for the above assumption. The susceptible plants, which may contain, in the surface lipids, arrestants to the insect became less suitable after the wax has been washed. Therefore, it is possible that the surface lipids of rice contain both arrestants and deterrents to the insect in different proportions and that the plant resistance is a result of the relative amounts of these compounds present in the wax. The role of surface lipids of ARC 6650 on the insect could not be demonstrated clearly in the present experiment. Furthermore, it was

also not possible to clearly demonstrate the influence of surface lipids of Bg 300 and Bg 379-2 on the insect.

Yield and composition of surface lipids

The lipid yield was estimated as a percentage of the fresh plant weight (figure 3). There were significant differences in the quantities of surface lipids of the test varieties. Resistant and moderately resistant varieties (ARC 6650, Ptb 33, Bg 379-2 and Bg 300) had more waxes than the susceptible ones (Bg 380 and TN1). Ptb 33 had the highest wax (0.25%) followed by ARC 6650 (0.2%), Bg 300 (0.16%), Bg 379-2 (0.1%), Bg 380 (0.07%) and TN 1 (0.08%). The resistant group had 3 times more wax than susceptible varieties. Among the moderately resistant varieties, Bg 300 had more wax than Bg 379-2.

For each variety, the amount of epicuticular wax (μg) extracted and the total area (cm^2) of the leaf sheaths used for the extraction were used to assess the amount of wax per unit area ($\mu\text{g wax}/\text{cm}^2$ leaf sheath surface) (figure 3). The figures thus obtained gave a rough estimate of the thickness of the surface wax for each cultivar. ARC 6650 and Ptb 33 had 150-12 $\mu\text{g}/\text{cm}^2$ as compared to Bg 380 and TN1 which had 35-40 $\mu\text{g}/\text{cm}^2$ of wax.

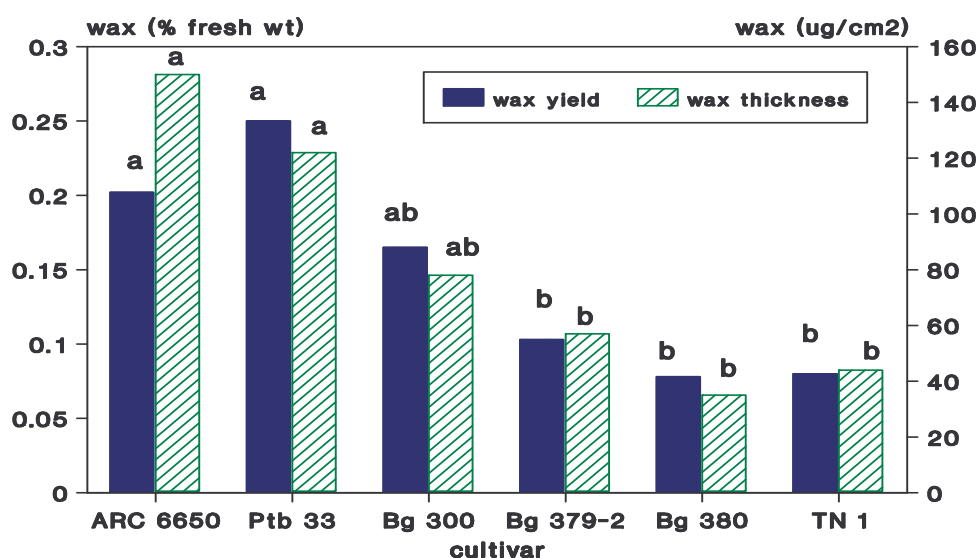


Figure 3. Amount of surface lipids as a percentage of fresh plant weight and estimated thickness of lipids. Columns with the same letter(s) are not significantly different at 5% level by DMRT

Fractionation of surface lipids:

The surface lipid extracts were separated according to the polarity by Thin Layer Chromatography (TLC). The lipid was fractionated into three distinct bands (R_f 0.7-0.9; 0.4-0.5; and 0.1-0.2). The bands were visible on the TLC plates when viewed under U.V. light. About 41-45% of the lipid extracts of Ptb 33 and TN 1 were fractionated into the non-polar

band (figure 4). The quantities fractionated into medium-polar and polar were relatively

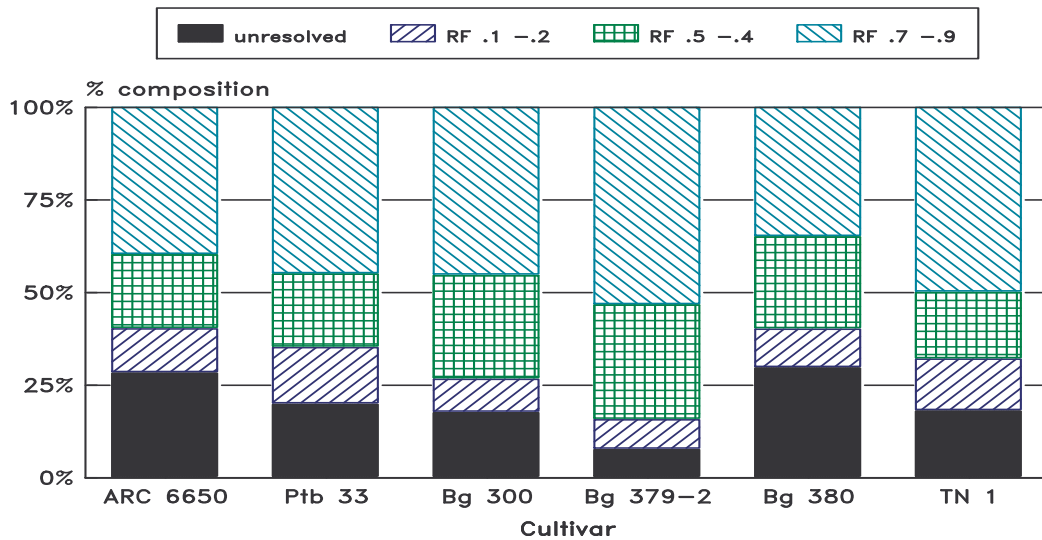


Figure 4. Estimated percentages of the different lipid fractions separated according to polarity.

smaller (35-30%) than the non-polar fractions. The relative quantities of the non-polar

(Rf 0.7-0.9), medium-polar (Rf 0.4-0.5) and more-polar (Rf 0.1-0.2) fractions did not differ among the cultivars. The percentages for the non-polar, medium-polar and polar fractions were respectively, 40-50%, 20-25% and 10-15%.

Feeding responses:

N. lugens gained 4-5 times more weight and excreted 2-3 times more honeydew on susceptible cultivars than on resistant cultivars (figure 5). The differences between resistant and susceptible cultivars were significant ($p < 0.05$).

Insects responded to Bg 300 (MR) as equally as a resistant cultivar. The correlation between weight gain and honeydew production was significant only for the susceptible and moderately resistant cultivars (figure 6). In general, across all test cultivars, the insect in 24 h produced 13 times less honeydew and lost 18% of their weight on para film wrapped plants than on normal plants. The insect's performances (both honeydew production and weight gain) on para film-wrapped cultivars were equally poor. These observations indicate the importance of the plant surface (including the surface wax and volatiles) for the insect to differentiate cultivars and to establish on them. When insects are deprived of direct contact with the plant surface they lost the ability to feed and to establish even on susceptible cultivars.

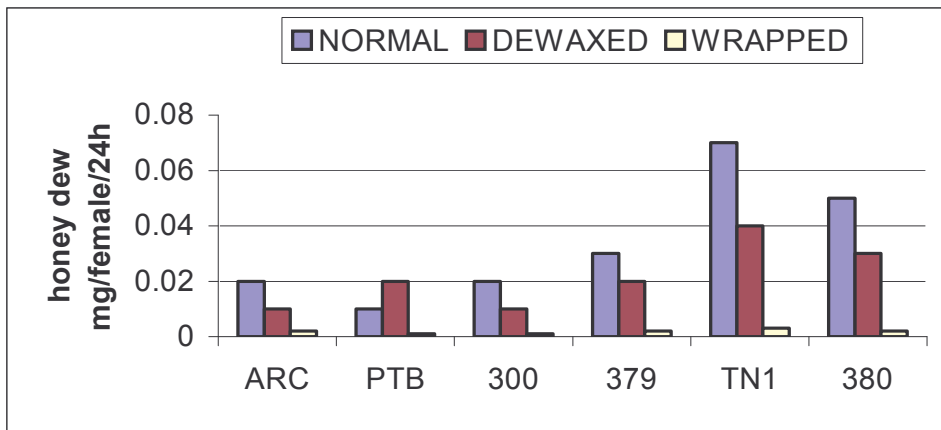
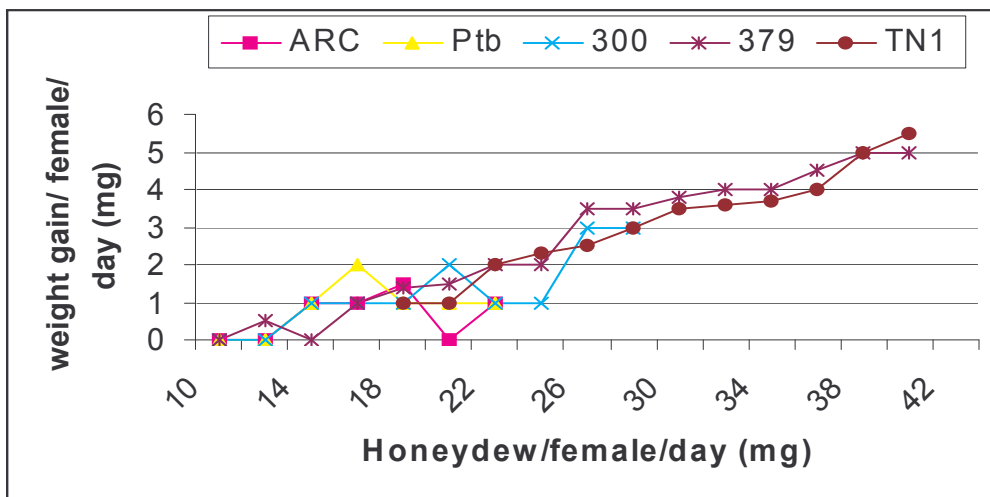


Figure 5. Weight gain and honeydew produced by BPH females on normal, de-waxed



and parafilm wrapped plants in 24h

Figure 6. Relationship between honey dew produced and weight gain by females in 24 h period.

On dewaxed susceptible cultivars the insect produced significantly more honeydew and gained more weight than on dewaxed resistant and moderately cultivars. The insect produced similar amounts of honeydew on dewaxed and normal plants of resistant and moderately resistant cultivars. But on susceptible cultivars the honeydew production was significantly lower on dewaxed plants than on normal plants. The percentage weight gain of the insect between normal and dewaxed plants differed only on Ptb 33 and Bg 300. It gained more weight on dewaxed Ptb 33 and Bg 300 than on normal plants.

These results provide further evidences on the interactions between the plant surface and the insect. It was clear that the insect's ability to locate phloem on the resistant Ptb33 and on moderately resistant cultivars was enhanced after the surface lipid was removed, and *vice versa* was true for the susceptible cultivars. It is therefore possible that specific compounds in the surface lipids of Ptb 33 and Bg 300 act as feeding deterrent to the insect. In contrast, the insect performed poorly on dewaxed susceptible cultivars (Bg 380 and TN1) as compared to

normal plants. They produced less honeydew on dewaxed plants, indicating that surface waxes of these cultivars are important for the insect to maintain normal feeding. Therefore, these observations suggest the surface waxes of rice contain both feeding stimulants and deterrents to the insect.

DISCUSSION

The plant surface is recognised as an important defence against insect attack and until it is penetrated by the insect the plant does not suffer damage (Chapman, 1977). The plant surface provides a broad structural and chemical diversity. Avoidance of herbivore damage is regarded as one of the evolutionary forces that may have influenced this diversity (Dillwith and Berberet, 1990). The defensive strategy of the plant surface against insects is considered under three headings; 1) effects at a distance, 2) effects when in contact and 3) effects when penetrating the surface (Southwood, 1986). Among the plant surface characteristics, trichomes and epicuticular waxes are considered important in studies dealing with insect-plant interactions.

Insects that are stimulated to settle on a plant first come in contact with the thin layer of surface lipids (epicuticular waxes) that covers the entire plant surface. Since the chemical composition of the surface lipids is distinctive for each plant species they could potentially be critical in host-plant selection by insects (Baker, 1982; Bianchi *et al.*, 1979).

Many aphids are known to respond to the epicuticular lipids of their host plants (Chapman and Bernays, 1989; Hisaaki *et al.*, 1989; Dillwith and Berberet, 1990). For instance resistance in marrow-stem kale to the cabbage aphid has been correlated with the reduced levels of lipids in the host plant (Dillwith and Berberet, 1990). Similarly the composition of surface lipids of winter wheat cultivars were found to be correlated with resistance to the grain aphid, *Sitobion avenae*, (Lowe *et al.*, 1985).

With regard to plant- and leaf- hoppers, however, the information available on the interaction with the surface lipids of their host plants is limited. The behavioural differences of *N. lugens* on some rice cultivars have been partly attributed to the differences in the chemical composition of the surface waxes of those cultivars (Cook *et al.*, 1987; Woodhead and Padgham, 1988). These authors further suggested that physical factors of the plant surface such as trichomes, surface silicification and epicuticular wax morphology are unlikely to influence such behavioral differences. For instance, the chemical composition of surface wax of IR 46 (a rice cultivar moderately resistant to *N. lugens*) was found to be responsible for the increased activity (or decreased acceptance) of *N. lugens* on that cultivar (Woodhead and Padgham, 1988).

The presence on the labial tip of *N. lugens* of several chemo- and mechano- receptors is a strong indication of the insect's ability to scan the plant surface prior to feeding (Foster *et al.*, 1983 a). However, the role of epicuticular waxes of rice on the behaviour of this insect has not been elucidated in detail.

Rice leaf wax contains n-alkanes (major chain lengths, C27, C29, C31 and C32), aldehydes (mainly of C30 and C32), esters (of C16 to C24 acids with C22 to C30 alcohols) and free alcohols (mainly of C28) (Bianchi *et al.*, 1979). It is also qualitatively similar to the leaf wax of maize seedlings but differs from those of wheat and sorghum due to the absence

of β -diketones and free acids (Bianchi *et al.*, 1979). The composition of rice wax, according to Uchiyama and Okuyama (1990), is follows: primary alcohols (20.4%), wax esters (27.8%), aldehydes (30.8%), and hydrocarbons (6.2%).

CONCLUSION

Parafilm, the standard membrane used in behavioural and feeding studies, contains a mixture of long chain alkanes similar to those found in plant waxes (Gaskin *et al.* (1971). As a result, many insects, including aphids and planthoppers, are known to adapt and recognise parafilm membrane as a substrate (Dillwith and Berberet, 1990). *N. lugens* also recognised parafilm and settled on plants wrapped with stretched parafilm and fed on food media offered behind parafilm membrane. However, the insect was less receptive to parafilm than the plant surface indicating that parafilm lacks the specific- feeding stimulants for *N. lugens*.

When presented with parafilm wrapped plants the insects made more movements and spent less time feeding than they did on normal plants. On dewaxed plants of resistant cultivars the insects moved less, spent more time feeding, gain more weight and excreted more honeydew than they did on normal plants indicating that surface wax of resistant plants make the insect less receptive to the plant. On the other hand, insect became less receptive to dewaxed plants of susceptible cultivars compared to normal susceptible plants. This indicates that some components of the surface wax of resistant cultivars are partly responsible for cultivar resistance to *N. lugens*. It is also clear that some surface wax components stimulate the insect to maintain continuous interaction with the plant, and on susceptible cultivars these components are more prevalent than on the resistant cultivars. Furthermore, a clear relationship between *N. lugens* resistance and surface wax content was observed, the more the amount of surface wax the more the plant was resistant to the insect.

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