Effect of Steam Distillate Extract of a Resistant Rice Variety on Feeding Behavior of *Nephotettix virescens* (Homoptera: Cicadellidae)

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**ABSTRACT**

*Nephotettix virescens* (Distant), a leafhopper pest of rice, is primarily a phloem feeder on plants of the susceptible rice variety 'Taichung Native 1' (TN1), but it becomes a xylem drinker on resistant 'ASD7' plants. The insect's feeding behavior on 'TN1' plants sprayed with the steam distillate extract of 'ASD7' plants was monitored with an electronic monitoring device and a lignin-specific dye. Application of 'ASD7' extract to susceptible 'TN1' plants disrupted the normal feeding behavior of the insect. Phloem feeding by the insect was significantly ($P < 0.05$) less on 'TN1' plants sprayed with 'ASD7' extract at 500, 1,000, 2,000, and 4,000 ppm than on control 'TN1' plants sprayed with acetone/water mixture. The reduced phloem feeding on the extract-treated plants was associated with a significant ($P < 0.05$) increase in probing frequency and an increase in duration of salivation and xylem drinking.

*Nephotettix virescens* (Distant), a leafhopper pest of rice in tropical Asia (Pathak 1968), damages susceptible rice plants severely by excessive feeding and transmission of several viral diseases. It damages resistant varieties less because the leafhopper's intake of phloem sap from such varieties is low and its drinking is restricted to xylem tissue (Auclair et al. 1982). Recently, we (Khan and Saxena 1984a) confirmed, by using a lignin-specific dye that is selectively translocated in xylem vessels, that *N. virescens* is primarily a phloem feeder on susceptible varieties, but on resistant varieties, it drinks mainly from xylem tissue. This shift from phloem feeding to xylem drinking on resistant rice is not well understood. Auclair et al. (1982) speculated that these differences were due to either the presence of a feeding deterrent in tissues adjacent to or within the sieve elements of resistant plants or the formation of callose or slime plugs in phloem in response to *N. virescens* probing. They stressed the need for precise identification of possible defense mechanisms against phloem-feeding leafhoppers and planthoppers.

Saxena (1978) and Okech (1981) demonstrated the role of volatiles in the rice plant's resistance to a phloem-feeding planthopper, *Nilaparvata lugens* (Stål). According to these authors, although the rice plant volatiles, extracted as steam distillates, were not likely to be found within the phloem tissue, their odoriferous and volatile nature strongly influenced the internal and external chemical environment of the rice plant and was thus ecologically significant in planthopper resistance. To determine the possible effects of plant volatiles on *N. virescens* feeding, we monitored its feeding behavior on plants of a susceptible rice variety 'Taichung Native 1' (TN1), sprayed with the steam distillate of the resistant rice variety 'ASD7' at different concentrations. The leafhopper's feeding behavior was monitored with a lignin-specific dye (Khan and Saxena 1984a) and a DC variant of an electronic recording device for insect feeding (Khan and Saxena 1984b).

**Materials and Methods**

Steam Distillation and Extraction of the Rice Plant. Leaves of 50-day-old plants of the resistant rice variety 'ASD7,' that had been grown in an insect-proof screenhouse, were harvested and ground with an electric grinder. Following the distillation and extraction method of Saxena (1973) and Okech (1981), a 200-g ground sample was steam-distilled for 3 h, during which time approximately 900 ml of distillate was collected. The distillate was extracted with diethyl ether (300 ml distillate: 100 ml diethyl ether) by thoroughly shaking a mixture of the two together in a separatory funnel for 5 min. Diethyl ether absorbed the essential oils and other volatiles and the mixture settled above the water layer in the funnel. The water layer was discarded while the ether extract was pooled in a 500-ml beaker, to which 100 g anhydrous sodium sulfate was added. The resultant mixture was kept inside a fume hood to evaporate excess ether and until the remaining volume was approximately 25 ml. The beaker then was covered with aluminum foil and held overnight to allow the sodium sulfate to absorb traces of water from the extract. The extract was evaporated fur-
ther to 10 ml and decanted into a preweighed glass vial, which then was covered with perforated aluminum foil and placed inside a desiccator. Ether was evaporated under vacuum, leaving behind a yellow oily residue. The vial was reweighed, sealed with nitrogen, and kept at −10°C. The residue was diluted in a mixture of acetone (four parts) and distilled water (one part), and tested at 500, 1,000, 2,000, and 4,000 ppm.

One h before being exposed to the insect, 15-day-old 'TN1' rice seedlings were sprayed individually with various concentrations of the steam distillate extract at a rate of 0.25 ml per plant with a quick-spray atomizer (Pierce Chemical Co., Rockford, Ill.). 'TN1' and 'ASD7' plants sprayed with an acetone/water mixture served as susceptible and resistant checks, respectively.

**Electronic Recording of Feeding Behavior.** The feeding activity of *N. virescens* on 'ASD7' extract-treated and control seedlings was recorded with a DC variant of an electronic monitoring device (Khan and Saxena 1984b). A 5-cm-long 18-μm gold wire (Tanaka Denshi, Kogyo, K.K., Tokyo, Japan) was attached with silver paint (Litsilber 200, Demetron, D-6450 Hanau, W. Germany) to the dorsum of an insectary-reared nonviruliferous 8- to 10-h-old female. The insect was starved (but water-satiated) for 2 h and then placed on the leaf blade of a treated or control plant. The gold wire was connected directly to the negative input terminal of a transistorized automatic and null-balancing DC chart recorder with a 250-mm recording width and an input resistance of 1 MΩ (Unicorder, Pantos, Nippon Denshi, Kagaku, Japan). The voltage source consisted of two 1.5-V DC batteries connected in series. The positive battery terminal was connected to the root of the rice plant through moistened filter paper and aluminum foil. The negative battery terminal was connected directly to the positive input terminal of the chart recorder. The recorder pen was adjusted to the chart baseline and insect feeding activity was monitored for 180 min at a chart speed of 1.5 cm/min with 500-mV amplifier power. Each treatment, including control, was tested 10 times using 10 new plants and 10 new insects. All recordings were made at 27 ± 2°C and 65 to 70% RH.

Waveforms recorded during leafhopper feeding were interpreted according to Chang’s (1978) identification of waveforms for probing, salivation, phloem feeding, and xylem drinking for the sugarcane leafhopper, *Perkinsiella saccharicida* Kirkaldy. For that species, voltage signals in xylem ingestion were identified as probe (P), phloem ingestion (Pi), probe (P), xylem ingestion (X), probe (P), and probe (P) for 40 s. The waveforms were electronically recorded during feeding of *N. virescens* females in batches of 10 were allowed to feed for 24 h on safranine-dyed seedlings sprayed with the steam distillate extract of the resistant 'ASD7' plants or with an acetone/water mixture. Insect excreta were collected on 9-cm-diameter filter paper disks placed around the base of seedlings. Red honeydew spots on the filter paper disks indicated xylem drinking by the leafhopper. They were counted to determine the extent of xylem drinking. To determine the extent of phloem feeding by the insect, filter paper disks were treated with a 0.1% ninhydrin acetone solution. Phloem feeding was quantified on the basis of the total area of bluish amino acid spots on filter paper disks. Each treatment, including controls, was replicated four times.

Data from both experiments were subjected to analysis of variance, and means were compared using Duncan’s (1951) multiple range test at the *P < 0.05* level.

**Results**

**Electronic Recording of Feeding Behavior.** Distinct differences were noted in the waveforms associated with *N. virescens* feeding activity on acetone/water-treated seedlings of susceptible 'TN1' and resistant 'ASD7' varieties (Fig. 1, Table 1). Phloem feeding was significantly (*P < 0.05*)

![Waveform Recording Diagram](image-url)
Table I. Means of electronically recorded events during 180-min feeding bouts by *N. virescens* females on 'TN1' rice plants sprayed with different concentrations of a steam distillate extract of 'ASD7'

<table>
<thead>
<tr>
<th>Extract conc&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Probes (no.)</th>
<th>Salivation (min)</th>
<th>Phloem feeding (min)</th>
<th>Xylem drinking (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>79b</td>
<td>12.8d</td>
<td>13.3b</td>
<td>12.5d</td>
</tr>
<tr>
<td>1,000</td>
<td>99a</td>
<td>16.8c</td>
<td>5.5c</td>
<td>20.9c</td>
</tr>
<tr>
<td>2,000</td>
<td>104a</td>
<td>17.7c</td>
<td>2.6cd</td>
<td>28.7b</td>
</tr>
<tr>
<td>4,000</td>
<td>116a</td>
<td>21.4b</td>
<td>1.8cd</td>
<td>36.9a</td>
</tr>
<tr>
<td>0 (susceptible check, 'TN1' seedlings)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30c</td>
<td>11.6d</td>
<td>34.4a</td>
<td>10.4d</td>
</tr>
<tr>
<td>0 (resistant check, seedlings)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>111a</td>
<td>24.3a</td>
<td>0.5d</td>
<td>22.7bc</td>
</tr>
</tbody>
</table>

Average of 10 replications, each replication using a new insect and a new plant. In a column, means followed by the same letter are not significantly different (*P* < 0.05; Duncan’s [1951] multiple range test).

<sup>a</sup> All concentrations prepared in a mixture of acetone and water (4:1).

<sup>b</sup> Sprayed with acetone/water mixture (4:1).

shorter on 'ASD7' seedlings (0.5 min) than on 'TN1' seedlings (34.4 min). In contrast, the mean durations of salivation and xylem ingestion on 'TN1' (11.6 and 10.4 min, respectively) were significantly (*P* < 0.05) shorter than those on 'ASD7' seedlings (24.3 and 22.7 min, respectively). Another notable difference was that *N. virescens* did significantly (*P* < 0.05) more probing while feeding on 'ASD7' plants (111) than on 'TN1' plants (30).

*N. virescens* shifted from phloem feeding to xylem drinking when allowed to feed on 'TN1' seedlings sprayed with 'ASD7' steam distillate extract (Fig. 1, Table 1). Phloem feeding was significantly (*P* < 0.05) longer on control 'TN1' plants than on 'TN1' treated with 'ASD7' extract. Phloem feeding on treated 'TN1' plants decreased progressively with application of increased concentrations of 'ASD7' steam distillate extract. The decrease in phloem feeding was accompanied by a corresponding increase in the frequency of probing, salivation period, and xylem drinking on treated plants.

**Lignin-specific Dye for Locating Feeding Sites.** On the basis of the number of red honeydew spots and the area of bluish amino acid spots, *N. virescens* proved to be a phloem feeder on control 'TN1' rice plants (Fig. 2a, Table 2), although occasionally, it also sucked small amounts of xylem sap (Fig. 2b). On resistant 'ASD7' plants, the hopper switched to xylem drinking, as indicated by a pre-

![Fig. 2. Filter paper disks on which *N. virescens* honeydew was collected when it fed on susceptible 'TN1' seedlings sprayed with acetone/water mixture (a and b); 'TN1' seedlings sprayed with 2,000 ppm (c and d); and 4,000 ppm (e and f) of steam distillate extract of resistant 'ASD7' plants; and 'ASD7' seedlings sprayed with acetone/water mixture (g and h). Bluish amino acid spots on ninhydrin-treated filter paper disks indicate phloem feeding (a, c, e, and g) and encircled honeydew droplets (b, d, f, and h) indicate xylem drinking.](image-url)
Table 2. Area of bluish amino acid spots indicating phloem feeding, and number of red honeydew droplets indicating xylem drinking by *N. virescens* on 'ASD7' steam distillate extract-treated 'TN1' or control rice seedlings

<table>
<thead>
<tr>
<th>Extract conc(b) (ppm)</th>
<th>Area of bluish acid spots(c) (mm(^2))</th>
<th>Red honeydew droplets(d) (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>805b</td>
<td>40c</td>
</tr>
<tr>
<td>1,000</td>
<td>481c</td>
<td>47c</td>
</tr>
<tr>
<td>2,000</td>
<td>390d</td>
<td>60b</td>
</tr>
<tr>
<td>4,000</td>
<td>431ed</td>
<td>82a</td>
</tr>
<tr>
<td>0(e) (susceptible check, 'TN1' seedlings)</td>
<td>Control</td>
<td>2325a</td>
</tr>
<tr>
<td>0(e) (resistant check, 'ASD7' seedlings)</td>
<td>Control</td>
<td>203e</td>
</tr>
</tbody>
</table>

In a column, means followed by the same letter are not significantly different (\(P < 0.05\); Duncan’s [1951] multiple range test).

* Average of 4 replications. In each replication, honeydew of 10 newly emerged females was collected on a 0.1-cm-diameter filter paper placed around the base of the seedlings. Red honeydew spots indicated xylem feeding on safranine-dyed seedlings and bluish amino acid spots indicated phloem feeding when filter paper disks were treated with a 0.1% ninhydrin/acetone solution.

\(b\) All concentrations prepared in a mixture of acetone/water (4:1).

\(c\) Sprayed with a mixture of acetone and water (4:1).


d ponderance of red honeydew spots (Fig. 2h). However, it also did some phloem feeding, as evidenced by the presence of traces of amino acids in the honeydew (Fig. 2g). The area of bluish amino acid spots on ‘TN1’ plants sprayed with ‘ASD7’ steam distillate extract (Fig. 2 c and e) was significantly \( (P < 0.05)\) smaller at all concentrations tested than on ‘TN1’ control plants. The decrease in phloem feeding on treated ‘TN1’ plants was accompanied by a significant \( (P < 0.05)\) increase in xylem drinking, as indicated by an increase in the number of red honeydew spots (Fig. 2 d and f).

**Discussion**

Steam distillate extract from the resistant ‘ASD7’ variety significantly affected the feeding behavior of *N. virescens*. We observed that the ‘ASD7’ extract, when sprayed on susceptible ‘TN1’ plants, imparted temporary resistance against the insect. Electronically recorded waveforms as well as the honeydew color in excreta of *N. virescens* have been put forth (Pathak et al. 1969, Auclair et al. 1982). Similar views were expressed by Oya and Sato (1981) to describe the varietal resistance in rice to another leafhopper, *Chilo suppressalis* (Walker), and the planthopper, *N. lugens*, and their implications for varietal resistance. Okech (1981) demonstrated that resistance or susceptibility of rice varieties to *N. lugens* was not due to the presence or absence of feeding deterrents in the phloem, but largely depended on the balance of allomones or kairomones predominant in rice plant volatiles.

The possible occurrence of one or more taste deterrents or the absence of feeding stimulants in the phloem sap of leafhopper-resistant varieties has been put forth (Pathak et al. 1969, Auclair et al. 1982). Similar views were expressed by Oya and Sato (1981) to describe the varietal resistance in rice to another leafhopper, *Nephotettix cincticeps* Uhler. However, our study demonstrates that external application of resistant rice plant volatiles to susceptible plants confers resistance against *N. virescens* without changing the chemistry of phloem sap of susceptible plants.

A possible reason for the shift in *N. virescens* feeding from phloem to xylem on resistant plants and on susceptible plants treated with extract from a resistant plant could be the harmful effects of its low molecular weight volatile chemical, which may penetrate the insect body through the cuticle or spiracles during feeding and respiration. Cuticular penetration by plant volatiles has been demonstrated in insects. For example, nicotine was detected in various tissues of the American cockroach, *Periplaneta americana* (L.), when the insect was exposed to nicotine vapor at 30°C (Ebeling 1964). Leafhopper and planthopper nymphs, particularly young instars, having a vestiture of poorly chitinized cuticle and relatively larger surface area because of smaller size, would be more vulnerable to volatile compounds of resistant plants while attempting to feed on them. For instance, Cheng and Pathak (1972) reported that only 0 to 3% of first-stage *N. virescens* nymphs reached the adult
stage on resistant varieties, whereas 76 to 90% of nymphs became adults on susceptible varieties. *N. virescens* increased drinking from xylem vessels on resistant varieties may be due to the insect's effort to eliminate harmful chemicals from its body. In hemipterans, drinking helps maintain a sufficiently large water turnover for the removal of toxins, where possibly the bulk of water flows through the cuticle (Stobbart and Shaw 1974). Thus, our findings seem to support Fraenkel's (1959, 1969) view that allelochmics play a vital role in insect/plant relationships, while potential nutritiveness of the host is a complementary factor.

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