Probing Behavior of Three Biotypes of *Nilaparvata lugens* (Homoptera: Delphacidae) on Different Resistant and Susceptible Rice Varieties

Z. R. KHAN1,2 AND R. C. SAXENA3

International Centre of Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya

**ABSTRACT** The feeding activity of biotype 1, biotype 2, and biotype 3 of *Nilaparvata lugens* (Stål) on differentially resistant rice varieties 'Taichung Native 1' ('TN1') (no resistance gene), 'Mudgo' (*Bph* 1 gene) and 'ASD7' (*bph* 2 gene), was monitored with a direct current variant of an electronic recording device. 'TN1' is susceptible to all three biotypes. 'Mudgo' is susceptible to biotype 2 but resistant to biotypes 1 and 3. 'ASD7' is susceptible to biotype 3 but resistant to biotypes 1 and 2. Electronically recorded waveforms corresponding to planthopper probing, salivation, and ingestion differed significantly on the leaf sheaths of susceptible and resistant rice plants. All three biotypes probed more readily and ingested longer on their respective susceptible varieties than on resistant varieties. The quantity of food ingested and assimilated also was significantly higher on susceptible than on resistant plants. Microsections of leaf sheath tissue were made to correlate stylet position and probing activities of the planthopper on susceptible and resistant rice varieties.

**KEY WORDS** Insecta, biotypes, feeding behavior, *Oryza sativa*

*Nilaparvata lugens* (Stål) is a destructive planthopper pest of rice in tropical and subtropical Asia. The insect damages susceptible rice plants directly as a result of its feeding and indirectly as a vector of ragged stunt and grassy stunt viral diseases. Planthopper feeding causes less damage to resistant rice varieties because sap intake by the insect from such varieties is reduced (Khan & Saxena 1984a, Velusamy & Heinrichs 1986). Nonetheless, the control of *N. lugens* through the use of resistant varieties has suffered a setback in recent years in several rice-growing countries because of development of insect pest biotypes (Saxena & Barrion 1984). These biotypes are represented by pest populations that differ in their ability to infest rice varieties with specific major genes for resistance.

Among the known *N. lugens* biotypes in the Philippines, biotype 1 represents the dominant field population that thrives only on those varieties that lack resistance genes. Biotype 2 can survive on and damage varieties carrying *Bph* 1 resistance gene and those susceptible to biotype 1. Biotype 3 can infest varieties possessing *bph* 2 resistance gene in addition to those susceptible to biotype 1. Although these biotypes can generally be separated from each other by assaying insect damage to differentially resistant rice varieties and by scoring plant damage in greenhouse and field trials (Seshu & Kaufman 1980, International Rice Research Institute 1982), more precise techniques are needed to identify biotypes based on their responses to plants of known genotypes. Because plant damage results from insect feeding, a technique that records subtle changes in *N. lugens* feeding responses to resistant and susceptible rice plants could be useful in the identification of biotypes as well as in the confirmation of host plant resistance. Studies of homopteran feeding are complicated by the fact that plant penetration by mouth parts and subsequent salivation and ingestion occur within plant tissues (McLean & Kinsey 1964, Triplehorn et al. 1984). An electronic method of monitoring the probing behavior of piercing-sucking insects was developed by McLean & Kinsey (1964) and later was used successfully to monitor probing activities of phytophagous and haematophagous insects (Kashin & Wakeley 1965, Smith & Friend 1970, Kennedy et al. 1978, Tarn & Adams 1982).

The technique already has been used to confirm resistance in several rice varieties to *Sogatella furcifera* (Horváth), a planthopper pest of rice (Khan & Saxena 1984b); *Nephotettix virescens* (Distant), a leafhopper pest of rice (Khan & Saxena 1985); and *N. lugens* biotype 3 (Velusamy & Heinrichs 1986).

Our study investigated the use of an electronic monitoring device for comparing the feeding behavior of the three *N. lugens* biotypes on differentially resistant rice varieties. To confirm further the results obtained by electronic monitoring, we measured the quantity of food ingested and assimilated by *N. lugens* biotypes on resistant and sus-
ceptible rice varieties. Microsections of leaf sheath tissue also were made to correlate stylet position and probing activities of *N. lugens* biotypes.

**Materials and Methods**

A direct current (DC) variant of an electronic monitoring system (Shaefers 1966; Khan & Saxena 1984b, 1985) was used to record the feeding activity of the three biotypes of *N. lugens* on differentially resistant rice varieties—"Taichung Native 1" ('TN1') (no resistance gene), 'Mudgo' (*Bph* 1 gene), and 'ASD7' (*Bph* 2 gene). 'TN1' is susceptible to all three biotypes, 'Mudgo' is susceptible to biotype 2 but resistant to biotypes 1 and 3, and 'ASD7' is susceptible to biotype 3 but resistant to biotypes 1 and 2.

To determine *N. lugens* feeding activity, a 10-cm-long, 10-μm gold wire (Tanaka Denshi, Kogyo, K. K., Tokyo, Japan) was attached by a small quantity of silver paint (Litsilber 200, Demetron, D-6450, Hanau, W. Germany) to the dorsum of an insectary-reared brachypterous female 8–10 h old. Before the wire was attached, the insect was anesthetized with carbon dioxide gas. The insect was starved but provided with water for 2 h and then placed on the leaf sheath of a 45-d-old 'TN1,' 'Mudgo,' or 'ASD7' plant. The gold wire was connected directly to the negative input terminal of a transistorized, automatic, null-balancing DC chart recorder having 250-mm recording width and input resistance of 1 MΩ (Unicorder, Nippon Denshi, Kagaku, Japan). The voltage source consisted of a 1.5-V penlight battery. The positive battery terminal was connected to the leaf sheath substrate through a moistened filter paper. The negative battery terminal was connected directly to the positive input terminal of the chart recorder. Insect feeding was recorded for 180 min at a chart speed of 1.5 cm/min. Each variety was tested 10 times for each biotype using fresh insects and fresh plants. All recordings were done at 27 ± 2°C and 70% RH.

The waveforms recorded during *N. lugens* feeding were compared and interpreted according to Khan & Saxena (1984b). The feeding site was marked with ink, and the plant tissue was dissected out for histological observations. Tissues were treated in the manner described by Willey (1971)—fixed in formaldehyde–acetic acid–alcohol (FAA) solution, dehydrated in a tertiary butyl alcohol series, sectioned crosswise at 15 μm thickness, and stained in safranin and fast green. Feeding tracks, including salivary sheath in leaf tissue, were photographed under a phase contrast microscope. Ten pieces of plant tissue from each variety were processed for microsections for each biotype.

For quantitative determination of food intake, brachypterous *N. lugens* females (8–10 h old) of biotypes 1, 2, and 3 were weighed individually and enclosed singly for feeding in parafilm (American Can Company, Greenwich, Conn.) sachets (5 by 5 cm) on the leaf sheaths of 45-d-old 'TN1,' 'Mudgo,' or 'ASD7' rice plants. After 24 h, insect weight and honeydew weight were determined separately on a microbalance (Mettler ME30). Control insects, starved but provided with water on a cotton swab, were maintained in identical parafilm sachets and were used to assess loss in body weight caused by normal metabolism and excretion. The amount of food ingested and assimilated by each female was calculated as follows (Saxena & Pathak 1977, Khan & Saxena 1984b):

\[
\text{Food assimilated} = W_1 \times [(C_1 - C_2)/C_1] + (W_2 - W_1),
\]

where *W*1 is the initial weight of the experimental insect, *W*2 is the final weight of the experimental insect, *C*1 is the initial weight of the control insect, and *C*2 is the final weight of the control insect. Food ingested was calculated as food assimilated plus weight of excreta. There were 10 replications for each variety and each biotype, including controls; each replication consisted of five females caged individually in parafilm sachets on five different plants.

Data were subjected to analysis of variance (ANOVA) and means were compared using Duncan's (1951) multiple range test at *P* = 0.05 in a 3 × 3 factorial experiment.

**Results**

Distinct waveforms related to planthopper probing, salivation, and ingestion were recorded during feeding of the three *N. lugens* biotypes (Fig. 1–3). The waveform patterns were common on all rice varieties tested. In all cases, insects ingested significantly less on resistant varieties than on susceptible varieties (Table 1). For instance, the feeding durations of biotype 1 females on resistant 'Mudgo' and 'ASD7' plants were significantly shorter than on susceptible 'TN1' plants. Biotype 2 females fed significantly longer on susceptible 'TN1' and 'Mudgo' than on resistant 'ASD7' plants. Ingestion periods of biotype 3 were significantly longer on susceptible 'TN1' and 'ASD7' plants than on resistant 'Mudgo' plants. In addition, the mean duration of salivation of the three biotypes was significantly longer on resistant varieties. Another notable indicator of resistance was an increase in the number of separate probes made by the three *N. lugens* biotypes during feeding on resistant plants.

A distinct undulate waveform, similar to the "A waveform" reported by Velusamy & Heinrichs (1986), was also recorded during *N. lugens* probing on susceptible and resistant rice varieties. The duration of the "A waveform" was significantly shorter during the insect’s feeding on susceptible varieties than on resistant ones. No honeydew droplets were excreted by the planthopper during the recording of "A waveform."

Microsections of rice leaf sheath tissue, in which a planthopper had probed and its feeding process was recorded, showed the terminal locations of the
feeding track always ending in phloem in each case where an ingestion or an "A waveform" was recorded on a susceptible or a resistant rice variety (Fig. 4a and b); however, the specific phloem tissue was extremely difficult to determine from these cross sections.

Quantitative determination of food intake and assimilation also confirmed that the three *N. lugens* biotypes ingested and assimilated significantly more on their respective susceptible rice varieties than on resistant ones (Table 2). Ingestion and assimilation of food by biotype 1 females was significantly reduced on 'Mudgo' and 'ASD7' plants compared with 'TN1' plants. Similarly, biotype 2 females fed and assimilated significantly more on 'TN1' and 'Mudgo' plants than on 'ASD7' plants. Food ingested and assimilated by biotype 3 females on 'TN1' and 'ASD7' plants was significantly higher than on 'Mudgo' plants.

**Discussion**

Plant resistance to insect pests depends largely upon the plant's ability to protect itself from successful feeding by the insect. Antixenosis or rejection of a plant host by a sucking insect is an im-

---

**Fig. 1.** Waveforms recorded during *N. lugens* biotype 1 probing on (a) 'TN1,' (b) 'Mudgo,' and (c) 'ASD7' rice varieties using an electronic monitoring device. Charts are to be read from right to left: P, probes; S, salivation; A, "A waveform"; I, ingestion.
important factor for resistance that can be measured by an electronic monitoring system (Tarn & Adams 1982). Electronic recording of probing behavior of plant-sucking insects is an easy and convenient technique that can be applied to a wide range of insects.

*Nilaparvata lugens* is primarily a phloem feeder on both resistant and susceptible rice varieties (Sogawa 1982, Khan & Saxena 1984a). When a plant-hopper probes into plant tissue, the plant-hopper's saliva and the plant sap, both electrically conductive, make it possible to include the feeding insect in an electrical circuit. In the present study, strip chart recordings showed that *N. lugens* biotypes respond to resistant plant varieties with increased probing frequency and salivation, resulting in reduced ingestion. Conversely, plant susceptibility was shown by rapid penetration, less salivation, and an increase in sustained ingestion. Reduced ingestion on resistant varieties was also confirmed by quantitative determination of planthopper food intake on resistant and susceptible rice varieties. Using an alternating current (AC) electronic monitoring system, Velusamy & Heinrichs (1986)
Fig. 3. Waveforms recorded during *N. lugens* biotype 3 probing on (a) 'TN1,' (b) 'Mudgo,' and (c) 'ASD7' rice varieties using an electronic monitoring device. Charts are to be read from right to left: P, probes; S, salivation; A, "A waveform"; I, ingestion.

reported similar feeding behavior of *N. lugens* biotype 3 on 10- and 40-d-old plants of susceptible and resistant rice varieties.

It is not clear whether the reduction in phloem ingestion by the planthopper on resistant rice varieties resulted from the lack of a phagostimulant or from the presence of a feeding deterrent or a repellent. Sogawa & Pathak (1970) reported that resistance of 'Mudgo' rice plants was caused by low concentrations of amino acids, particularly asparagine, which in higher concentrations stimulates *N. lugens* feeding. Later, Shigematsu et al. (1982) reported the presence of β-sitosterol, a sucking inhibitor, in resistant rice varieties. Recently, Saxena & Okech (1985) reported that resistance or susceptibility of rice varieties to *N. lugens* was due not to the presence or absence of feeding deterrents in the phloem, but to the balance of allomones or kairomones predominant in rice plant volatiles. Khan & Saxena (1986) also demonstrated the important role of rice plant volatiles in imparting resistance to *S. furcifera.*

No direct evidence demonstrates the relationship between waveforms obtained from AC and DC...
Fig. 4. Cross sections of (a) a 'TN1' rice leafsheath and (b) an 'ASD7' rice leafsheath containing *N. lugens* biotype 1 feeding tracks. Bodies identified are: FT, feeding track; MX, metaxytem; PH, phloem; PX, protoxylem. The planthoppers that produced ingestion (I) waveform on 'TN1' plant (a), and "A waveform" on 'ASD7' plant (b), ended their feeding tracks (FT) in the phloem (PH).

Electronic monitoring systems; however, using a DC variant of the monitoring system, we recorded a distinct waveform similar to the "A waveform" reported by Velusamy & Heinrichs (1986) during *N. lugens* feeding on rice plants. We recorded the insect's feeding track ending in the phloem when the planthopper produced the "A waveform," but we did not observe any excretion of honeydew during that period. Velusamy & Heinrichs (1986) speculated that this waveform may be related to the sensory response that provides the insect with information regarding the suitability of phloem sap for ingestion. This waveform can be used as a parameter, in addition to the ingestion period, for measuring resistance or susceptibility of rice varieties because its duration is significantly longer on resistant varieties than on susceptible ones.

In addition to other established screening procedures, the electronic recording of the probing activity of *N. lugens* biotypes can be useful in evaluating and confirming resistance in rice germplasm. The technique has been used to study the differential probing responses of four biotypes of the spotted alfalfa aphid, *Theroaphis maculata* (Buckton), on resistant and susceptible alfalfa clones (Nielsen & Don 1974). The electronic monitoring of insect probing has also facilitated confirmation of host resistance in various crop plants such as sugar beets against green peach aphid, *Myzus persicae* (Sulzer) (Haniotakis & Lange 1974); muskmelon against melon aphid, *Aphis gossypii* Glover (Kennedy et al. 1978); sorghum against green bug, *Schizaphis graminum* (Rondani) (Campbell et al. 1982); and rice against *S. furcifera* (Khan & Saxena 1984b), *N. virescens* (Khan & Saxena 1985), and *N. lugens* (biotype 3) (Velusamy & Heinrichs 1986).

To deter the selection and spread of *N. lugens* biotypes, varieties with stable resistance are needed. The electronic monitoring system can be valu-

Table 1. Means of various electronically recorded events during 180-min feeding bouts by females of the three biotypes of *N. lugens* on resistant and susceptible rice varieties

<table>
<thead>
<tr>
<th><em>N. lugens</em></th>
<th>'TN1'</th>
<th>'Mudgo'</th>
<th>'ASD7'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probes (no.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotype 1</td>
<td>9 ± 2.7az</td>
<td>37 ± 7.7by</td>
<td>49 ± 8.1ax</td>
</tr>
<tr>
<td>Biotype 2</td>
<td>11 ± 4.4ay</td>
<td>37 ± 7.0cy</td>
<td>43 ± 11.6ax</td>
</tr>
<tr>
<td>Biotype 3</td>
<td>16 ± 6.2ay</td>
<td>53 ± 16.7ax</td>
<td>13 ± 5.9by</td>
</tr>
<tr>
<td>Salivation (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotype 1</td>
<td>18.5 ± 2.4az</td>
<td>51.8 ± 9.9ax</td>
<td>43.7 ± 11.3by</td>
</tr>
<tr>
<td>Biotype 2</td>
<td>13.7 ± 3.9ay</td>
<td>20.1 ± 7.3cy</td>
<td>57.3 ± 10.1ax</td>
</tr>
<tr>
<td>Biotype 3</td>
<td>23.1 ± 8.7ay</td>
<td>39.6 ± 5.3bx</td>
<td>17.7 ± 4.5cy</td>
</tr>
<tr>
<td>A waveform (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotype 1</td>
<td>13.6 ± 4.1ax</td>
<td>42.8 ± 6.7by</td>
<td>54.3 ± 7.4ax</td>
</tr>
<tr>
<td>Biotype 2</td>
<td>14.1 ± 1.7ay</td>
<td>11.5 ± 1.5cy</td>
<td>49.7 ± 4.4ax</td>
</tr>
<tr>
<td>Biotype 3</td>
<td>9.7 ± 1.3bx</td>
<td>59.9 ± 8.3ax</td>
<td>14.3 ± 2.4by</td>
</tr>
<tr>
<td>Ingestion (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotype 1</td>
<td>52.7 ± 8.2ax</td>
<td>9.7 ± 1.8bx</td>
<td>16.3 ± 2.6cy</td>
</tr>
<tr>
<td>Biotype 2</td>
<td>64.5 ± 6.9ax</td>
<td>59.7 ± 8.7ax</td>
<td>19.7 ± 4.2by</td>
</tr>
<tr>
<td>Biotype 3</td>
<td>49.5 ± 4.8bx</td>
<td>13.4 ± 2.9by</td>
<td>57.3 ± 5.2ax</td>
</tr>
</tbody>
</table>

Means (±SD) followed by a common letter within a column (a-c) and within a row (x-z) are not significantly different (P = 0.05, Duncan's [1955] multiple range test). The data were analyzed in a 3 × 3 factorial design. The results showed a highly significant interaction between rice variety and insect biotypes. Main effects of rice variety and of insect biotypes were also significant. Average of 10 replications, each replication using a new plant and a new insect.
Table 2. Quantity of food ingested and assimilated by females of the three biotypes of *N. lugens* on different resistant and susceptible rice varieties

<table>
<thead>
<tr>
<th><em>N. lugens</em></th>
<th>'TN1'</th>
<th>‘Mudgo’</th>
<th>‘ASD7’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotype 1</td>
<td>29.31 ± 4.30</td>
<td>2.94 ± 0.04</td>
<td>3.11 ± 0.06</td>
</tr>
<tr>
<td>Biotype 2</td>
<td>33.43 ± 6.21</td>
<td>31.35 ± 3.29</td>
<td>2.37 ± 0.13</td>
</tr>
<tr>
<td>Biotype 3</td>
<td>27.49 ± 3.48</td>
<td>3.71 ± 0.10</td>
<td>30.16 ± 5.11</td>
</tr>
</tbody>
</table>

| Biotype 1   | 1.35 ± 0.13 | 0.99 ± 0.01 | 0.11 ± 0.03 |
| Biotype 2   | 1.27 ± 0.11 | 1.41 ± 0.05 | 0.15 ± 0.04 |
| Biotype 3   | 1.29 ± 0.10 | 1.08 ± 0.02 | 1.35 ± 0.05 |

Means (±SD) followed by a common letter within a column (a, b) and within a row (x, y) are not significantly different (*P* = 0.05, Duncan’s [1951] multiple range test). The data were analyzed in a 3 × 3 factorial design. The results showed a highly significant interaction between rice variety and insect biotypes. Main effects of rice variety and of insect biotypes were also significant. Average of 10 replications, each replication using five females caged individually on five different plants.

References Cited


1984b. Electronically recorded waveforms associated with the feeding behavior of *Sogatella furcifera* (Homoptera: Delphacidae) on susceptible and resistant rice varieties. J. Econ. Entomol. 77: 1479-1482.


Tarn, T. R. & J. B. Adams. 1982. Aphid probing and

Acknowledgment

This paper is a part of collaborative research with the International Rice Research Institute (IRRI), P.O. Box 933, Manila, Philippines.

Able in further examining selected varieties from mass screening programs. The technique is also of value in investigating the nature of plant resistance.


Received for publication 13 July 1987; accepted 17 May 1988.