Mechanism of Varietal Resistance to the Rice Green Leafhopper (*Nephotettix cincticeps* Uhler)*

By SUSUMU KAWABE**

Rice Research Division, Tohoku National Agricultural Experiment Station
(Omagari, Akita, 014-01 Japan)

Nephotettix cincticeps Uhler is one of several species commonly called the rice green leafhoppers, which are very detrimental to rice production and distributed from temperate to tropical zones. The rice green leafhoppers cause direct damage by sucking plant sap, and indirect damage by transmitting virus and MLO (mycoplasma like organism) diseases.

The present study was conducted for seven years till 1980 to elucidate mechanisms governing the resistance of rice varieties to *Nephotettix cincticeps*, with special reference to its feeding behavior.

Characteristics of resistance to the rice green leafhopper

Rice varieties resistant to *N. cincticeps* carry all three characteristics of insect resistance,[1,9,10,12] namely, antibiosis, antixenosis[11] and tolerance. Effects which are expressed by the resistance of rice plants on the leafhoppers and rice plants themselves are as follows (Fig. 1).[3]

When the leafhoppers were reared on resistant plants, they developed very slowly, died quickly and laid no eggs. The first instar nymphs died within two days on leaf sheaths of resistant grown-up rice plants. If the adults emerged on resistant plants, their size was considerably smaller than those of normal adults emerged on susceptible plants.

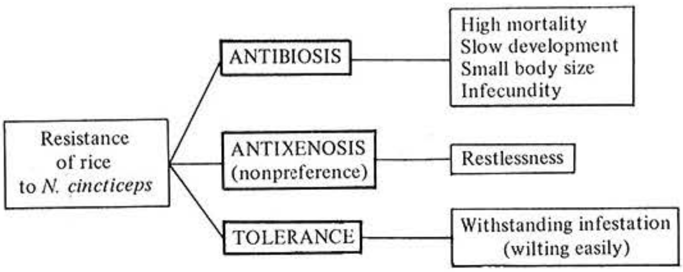


Fig. 1. Multilateral effects of the rice green leafhopper resistance

The leafhoppers do not settle down on resistant plants, but they continue to move until they can settle on susceptible plants.

Both susceptible and resistant plants were injured by removal of their plant sap. However, the resistant plants could grow better

* Summarized from the doctor thesis (Univ. Tokyo, 1980) titled "Studies on the mechanism of rice resistance to the rice green leafhopper, *Nephotettix cincticeps* Uhler", placing emphasis on the feeding behavior.

Present address:
** First Environment Division, Kyushu National Agricultural Experiment Station
(Chikugo, Fukuoka, 833 Japan)

than the susceptible ones under infestation, although resistant seedlings wilted more severely than susceptible ones depending on water condition. Resistant plants are also affected by the leafhoppers inserting stylets into the plant tissue and sucking plant sap.

Qualitative and quantitative analysis of excreta on resistant and susceptible plants

To know whether quantities and components of plant sap ingested by the rice green leafhopper are different between resistant and susceptible plants, droplets of honeydew excreted by the leafhoppers on resistant and susceptible seedlings of rice were collected.[3] This was done by rearing the leafhoppers in plastic containers under controlled conditions. Droplets on the inner wall and on the plastic plate were collected with a filter paper and analysed. The quantity of the excreta was shown by measuring the weight increase of the filter paper used to wipe up all droplets of excreta, and sugar content of the excreta was determined colorimetrically using the phenol-sulfuric acid method. The results when the leafhopper was reared on susceptible (Towada and Yugo) and resistant (Tadukan and Lepedumai) varieties are shown in Fig. 2.

The honeydew excreted on resistant rice plants was by far more in quantity than those on susceptible plants. On the other hand, the honeydew excreted on the resistant plants contained no sugars, although those from the susceptible plants contained about 1 or 2% sugars. Plate 1 shows the TLC chromatogram of the excreta. In this case, the excreta of the leafhoppers on the resistant plants were spotted in quantity ten times more than the excreta on the susceptible plants. However, no sugar was detectable in the chromatogram of the excreta of leafhoppers on the resistant plants. The differences in quantities and chemical compositions of the excreta between resistant and susceptible plants suggested that feeding behavior on resistant plants differed from that on susceptible plants.

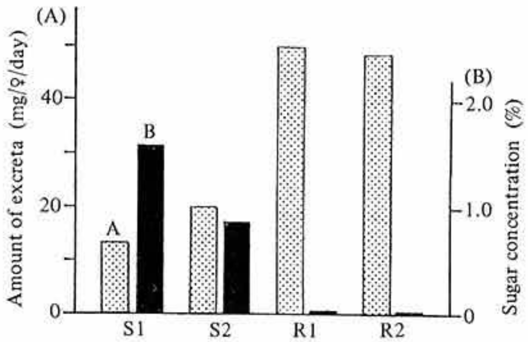


Fig. 2. The amount and sugar concentration of honeydew excreted on susceptible and resistant rice plants
80% ethanol-extract from a filter paper which had absorbed honeydew was concentrated by evaporation and diluted with water properly for analysis.
Susceptible varieties: S1, Towada; S2, Yugo
Resistant varieties: R1, Tadukan; R2, Lepedumai
A, amount of excreta; B, sugar concentration in excreta

The results of sugar analysis of honeydew excreted on 34 varieties of rice are shown in Table 1.

Droplets excreted on 12 resistant varieties contained no sugars whereas on all 14 susceptible varieties droplets contained sugars. Of 8 semi-resistant varieties, droplets on 5

Table 1. Presence of sugars in honeydew excreted on susceptible, medium and resistant rice varieties

Variety	No. varieties	No. varieties of each conc.		
		++	+	−
Resistant	12	0	0	12
Medium	8	1	4	3
Susceptible	14	13	1	0

Presence of sugars was analysed qualitatively using thin-layer chromatography; see the legend to Plate 1. Honeydew droplets were collected with pipettes and spotted directly on the thin-layer plate.
++ Sugars were contained.
\+ Sugars were in low concentration.
− No sugar was detected.

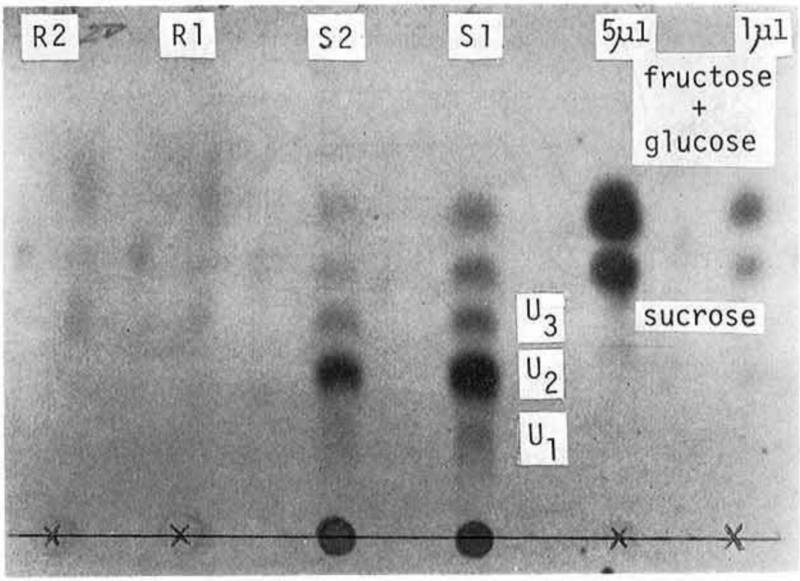


Plate 1. Thin-layer chromatogram of sugars in honeydew excreted on susceptible and resistant rice plants

As for varieties and preparation of sample excreta, see the legend to Fig. 2. Thin-layer plate; Kiesel-gel 60 (Merck 0.25 mm), separation: butanol-acetic acid-water (4:1:1), visualization; anisaldehyde-sulfuric acid reagent. RSM; reference sugar mixture containing 0.1% sucrose, glucose and fructose; 1μl and 5μl of the mixture was spotted. One μl of sample excreta (in terms of original excreta) from susceptible plants and 10μl of sample excreta from resistant plants were spotted. U_1, U_2 and U_3 are unknown sugars or sugar derivatives.

varieties contained some sugars. The lack of sugar in excreta was regarded as a phenomenon linked with resistance itself.

Clarification of resistance mechanism by electronic measurement of insect feeding behavior (EMIF)

Therefore, the differences of the feeding behavior on the resistant and susceptible plants were investigated in detail by means of electronic measurement of insect feeding system, called the EMIF system. The EMIF system was developed by Mclean and Kinsey originally in 1964, using aphid as test insects.[13,14,15,16] In order to apply EMIF to our study, modification of the system were necessary.[2,4,5,8]

Two basic modifications were the addition of a variable oscillator and an amplifier with both linear and logarithmic functions. In addition, the new system has two channels so that two individual insects can be monitored simultaneously on two separate substrates. A marker is attached to the recorder. Therefore, whenever droplets were excreted, the observer can mark on the chart by punching the button.

A 500 hz, 2-volt, alternating current source was commonly applied the plant on which feeding behavior of the leafhopper is to be examined. This frequency higher than an ordinary current source eliminated disturbance of the insects caused by electronical potential and reduced problems of electrical noise from external sources. The high current flow during salivation caused the recorder pen to over-shoot, but the logarithmic amplifier attenuated the higher amplitude peak

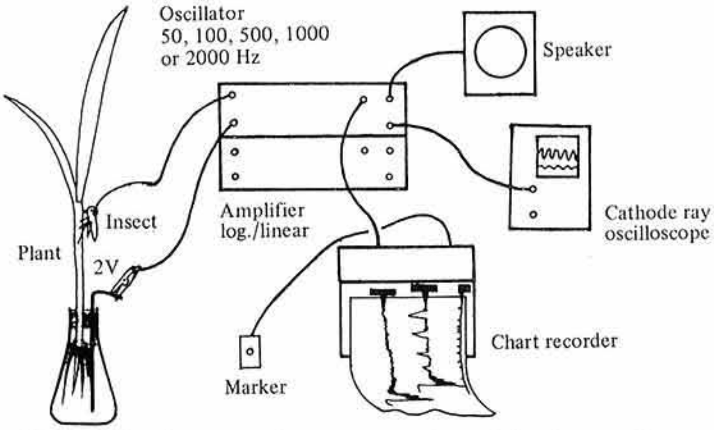


Fig. 3. A diagrammatic representation of the improved system of EMIF and a leafhopper probing a rice plant, showing the points of attachment to the system

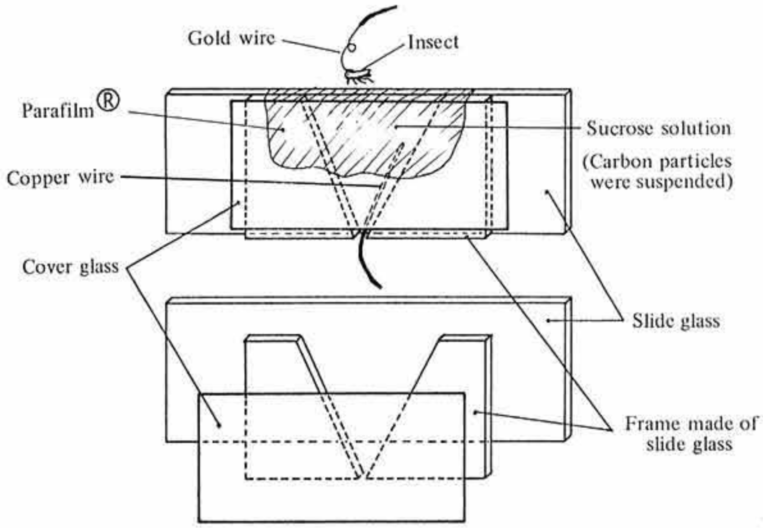


Fig. 4. Observation chamber which is built easily
Three pieces of slide glass and a piece of cover glass are sticked together with an adhesive agent, and the upper opening is covered with a slip of Parafilm® stretched out. A copper wire is inserted through the lower small opening so that a 2.0 v_{p-p} potential from the oscillator is applied to the sucrose solution.

during salivation.

The diagram given in Fig. 3 shows a leafhopper probing a rice plant, and points of attachment of EMIF system. A length of diameter 20 μm gold wire was attached to the dorsum of the rice green leafhopper. The wired leafhopper was given access to a rice seedling. A copper stick wired to the alternating current source was inserted in a flask filled with water to which roots of the seedling were

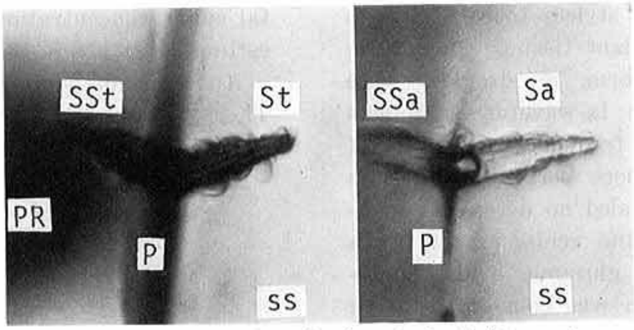


Plate 2. A stylet bundle inserted (left) and a salivary sheath left after the stylets was drawn out (right) in the observation chamber

St, a stylet bundle; Sa, a salivary sheath; PR, a probosics; P, Parafilm® stretched out; ss, sucrose solution; SSt, a shadow of the stylet bundle; SSa, a shadow of the salivary sheath

submerged.

The feeding behavior on the observation chamber was also measured by EMIF (Fig. 4). This was done to elucidate the basic relationships between the feeding behavior and waveforms recorded on the strip-chart by EMIF system.

The triangular chamber was filled with 5% sucrose solution. Carbon particles of Chinese ink were suspended in the solution. A wire was placed in the solution so that voltage could be applied. The chamber was then placed on the stage of a compound microscope, and stylet of activities and the flow of carbon particles in the solution could be observed. The stylet bundle inserted into sucrose solution in the observation chamber is shown in Plate 2. A salivary sheath is formed around the stylet bundle and left in the sucrose solution after the stylets is withdrawn.

Four typical waveforms were recorded by the EMIF of the leafhopper while probing the rice plant (Fig. 5). The insect pierced plant tissue with a stylet bundle, salivated, and ingested plant sap. Salivation-, Ix- and R-waveforms were also recorded when the insect probed the sucrose solution. But Ip-waveform was recorded only when the leafhopper probed rice plants.

R-waveform was recorded when the insect

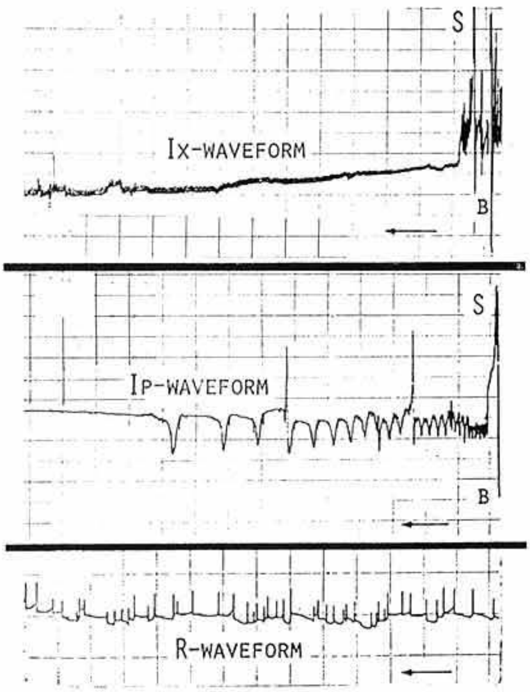


Fig. 5. Waveforms recorded by EMIF when the rice green leafhopper was probing rice plants

S, salivation-waveform; B, initiation of a probe.

Charts to be read from right to left. Time between vertical chart lines is two min.

was resting with its stylets inserted into a sucrose solution or plant tissue. During recording the R-waveform, no droplets were excreted. During the Ix-waveform, droplets were usually excreted frequently, at a rate of several droplets or more per min. Analysis of these droplets revealed no detectable presence of sugar or amino acids, except for a low concentration of glutamic acid. Therefore, the Ix-waveform was considered to be recorded when leafhoppers were ingesting fluid from xylem vessels.

The leafhoppers producing the Ip-waveform excreted droplets at a rate of one droplet or less per min. The excreted droplets accompanied with the Ip-waveform contained a high concentration of sugars and many kinds of amino acids. In Plate 3, three droplets which fell on TLC plate when the Ip-waveform was being recorded were developed and visualised with anisaldehyde sulfuric acid reagent. Total sugar concentration of these droplets was estimated at about 15%.

Plate 3. Thin-layer chromatogram of honeydew excreted during Ip-waveform
Three droplets excreted were caught directly onto the thin-layer pplate. As for TLC, see the legend to Plate 1.

Amino acids were analysed by TLC, too. There were almost all amino acids which were considered to exist in phloem sap (Plate 4).

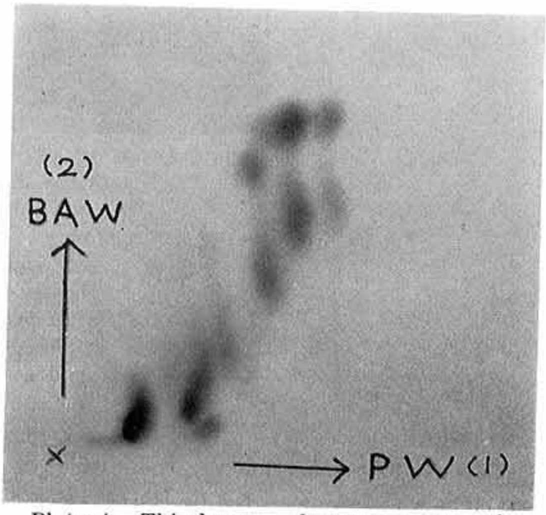


Plate 4. Thin-layer chromatogram of amino acid in honeydew excreted during Ip-waveform
Thin-layer plate; Kieselgel 60 (Merck 0.25), two-dimensional separation; (primary) phenol-water (3:1) and (secondary) butanol-acetic acid-water (4:1:1), visualization; ninhydrin solution in ethanol. A concentrated sample honeydew was used for analysis.

Fig. 6. A refinement in Ix-waveform
The speed of the strip-chart recorder was increased to 60 cm/min from 1 cm/min of ordinary measurement.

Accordingly it was concluded that the Ip-waveform showed sap ingestion from sieve elements of the phloem.

A refinement in the Ix-waveform is shown in Fig. 6, where fast ingestion produced more regular waves than slow ingestion. The rate of ingestion is dependent upon activity of the cibarial pump in the mouth and observed as a speed of carbon particles flowing toward the tip of the stylet bundle.

Then the difference between the feeding behavior of the leafhopper on resistant plants and that on susceptible plants was investigated with the aid of EMIF system. On resistant plants durable Ip-waveform was not recorded, whereas TIp-waveform was recorded, and the latter bears a strong resemblance to the initial stages of the Ip-waveform (Fig. 7).

The period that the TIp-waveform was produced was too short for the insect to ingest enough sap to excrete droplets. Therefore, to determine what tissue the stylets reached during this period, a histological method was used. Observation of paraffin-microsection of plant tissue probed by the insect showed that the salivary sheath reached the sieve elements of the resistant plant when TIp-waveform was produced (Plate 5). It was proved that the insect pierced the sieve elements but withdrew the stylets without ingesting phloem sap on the resistant plant. It was surmised from these results that the sieve elements of the resistant plant contain a feeding deterrent.

The graph in Fig. 8 shows each feeding activity on susceptible, semi-resistant and resistant plants. Each bar represents the mean percent of time engaged in each activity in one day. The most critical differences were in the existance and the duration of the Ip-waveform, which was absent in the resistant plant, weak in the intermediate plant and pronounced in the susceptible one. Beside the period engaged in each activity, the number of droplets excreted during the period of showing each waveform was also counted to know the amount of sap ingested. Although the mean time spent in ingestion of xylem sap on each variety was not significantly different, the amount of xylem sap ingested on the resistant plant was several times larger than on the susceptible one, due to a much higher ingestion rate.

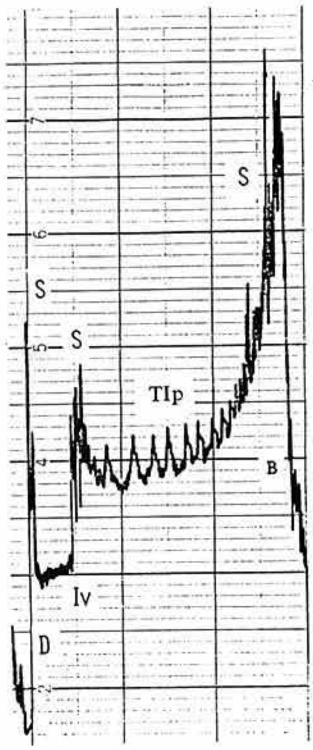


Fig. 7. A TIp-waveform produced on a resistant rice plant
B, start of probing; S, salivation; D, withdrawal of stylets from the plant. The The TIp- (trial ingestion of phloem sap) waveform bears a strong resemblance to the initial stage of the Ip-waveform on susceptible plants.

The graph describing the results on the susceptible rice plants is that of normal feeding behavior. The insect ingests both phloem and xylem sap. The amount of phloem sap ingestion is far less than that of xylem sap. The period of ploem sap ingestion is however longer than xylem sap ingestion, showing that a kind of inverted relationship exists.

Conclusion

The conclusion of this study is represented in Fig. 9. Inhibition of phloem sap ingestion

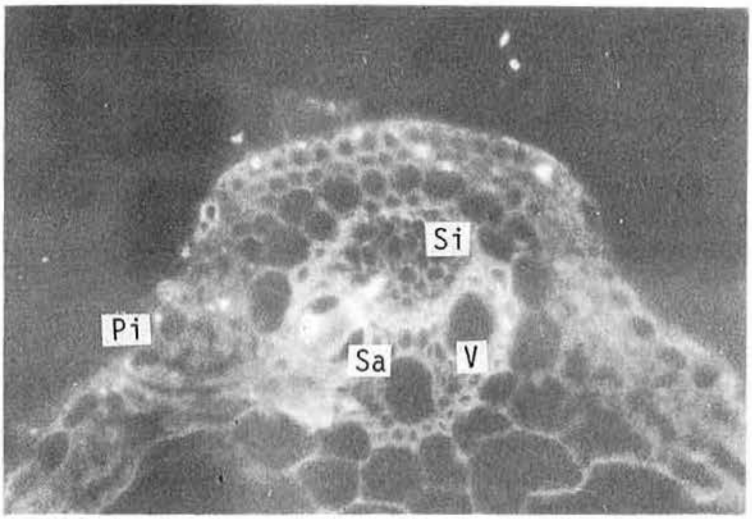


Plate 5. A salivary sheath reaching phloem sieve elements
The salivary sheath remained in the tissue of the resistant plant after a rice green leafhopper made a probe accompanied with the TIp-waveform. S; salivary sheath, Si; sieve elements, V; vessel, Pi; a piercing passage. (Ando, Y., & Kawabe, S., unpublished)

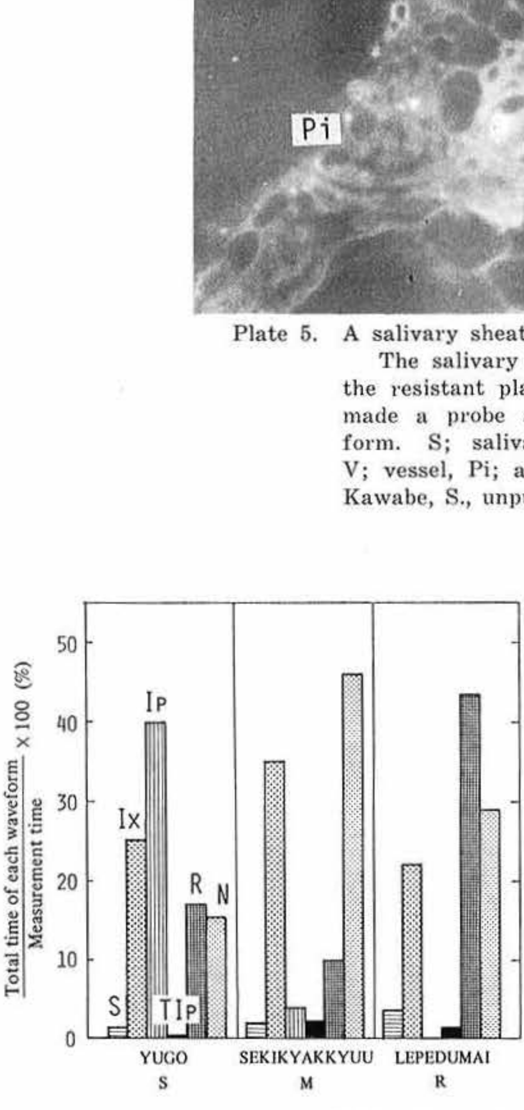


Fig. 8. Mean percentage of time engaged in each of the activities to ca. 24 hr access period shown by the rice green leafhopper on susceptible, medium and resistant rice plants
As for waveforms and feeding activities, see Figs. 5 & 7 and the text. N: Not probing.

is the basis of all phenomena related to the rice varietal resistance to the leafhopper, *Nephotettix cincticeps*. We suggest that inhibition is derived from a feeding deterrent contained within the sieve elements of resistant plants.[3]

Actually this figure closely resembles other types of plant resistance to various homopterous insects including aphids, planthoppers, etc.

To follow up the hypothesis of a feeding deterrent, we developed a method for collecting phloem sap.[6,7] The diagram in Fig. 10 shows how to collect pure sap from the phloem sieve elements using a YAG, Yttrium Aluminum Garnet, laser beam. The laser beam severed accurately the stylets bundles of the leafhopper and the brown planthopper, *Nilaparvata lugens*, during their process of feeding (stylectomy). Rice phloem sap exuded from the cut end of stylets embedded in the phloem sieve elements. Thus, pure phloem sap of rice could successfully be collected by stylectomy for the first time for analysis.

Finally, an experiment to confirm whether phloem sap from resistant plants contained a

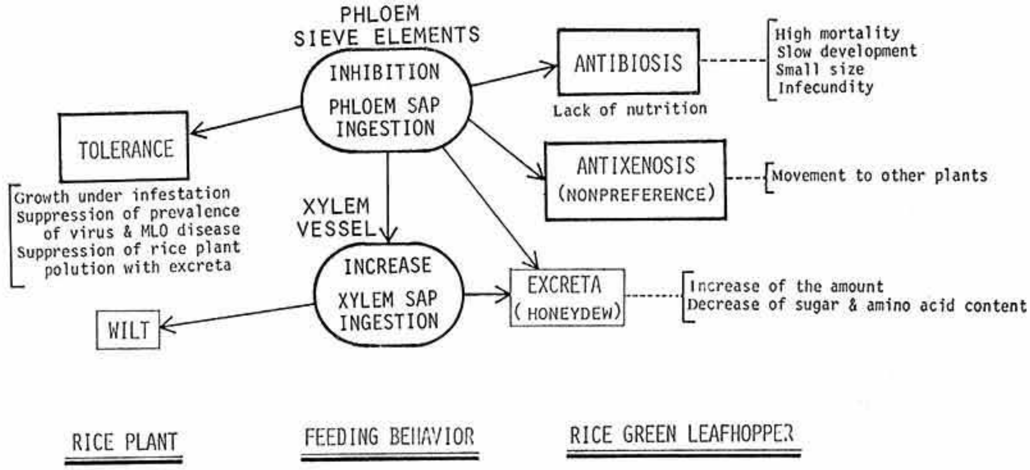
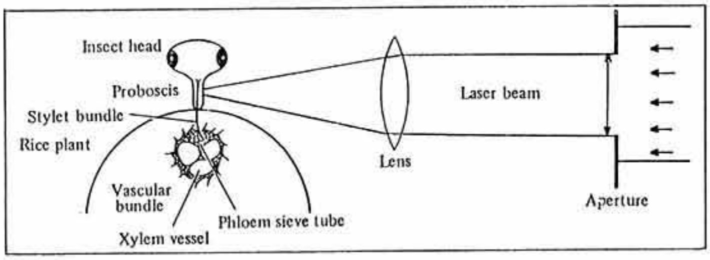
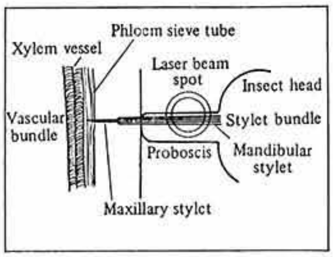


Fig. 9. Mechanism of varietal resistance to the rice green leafhopper with an inhibitor to phloem sap ingestion as the central figure



1. Diagrammatic representation of a cross section of the rice plant, the insect head, and YAG laser beam focused on proboscis for stylectomy.



2. Diagrammatic representation of a longitudinal section of the rice plant, the insect mouthparts showing the proboscis, the stylets, and the laser beam spot. The stylets reach the sieve tube.

Fig. 10. Stylectomy using a YAG laser to collect rice phloem sap[6)]

feeding deterrent was conducted but unsuccessful yet. The leafhopper ingested the phloem sap of resistant plants as did that of susceptible plants, contrary to our expectation. The insect was considered to withdraw its stylets from the phloem sap soon after obtaining a foretaste of that, because the TIp-waveform was observed only for a short time on resistant plants. A biochemical or physiological mechanism (e.g., phytoalexin, mechanical barrier, etc.) may possibly exist in phloem sieve elements to prevent the leafhopper from ingesting sap there in the resistant plants other than a stable feeding deterrent. Anyhow, a detailed chemical analysis is under way.

References

1) Inoue, H.: Larval development of the green rice leafhopper, *Nephotettix cincticeps* Uhler, and the white back planthopper, *Sogatella furcifera* Horváth, on Japonica and Indica rice varieties. *Ōdōkon Chugoku*, 8, 17–19 (1966) [In Japanese].
2) Kawabe, S.: Electronic measurement of insect feeding behavior (EMIF). *Shokubutsubōeki*, 33, 65–70 (1979) [In Japanese].
3) Kawabe, S.: Feeding behavior of *Nephotettix cincticeps* and resistance of rice. *Shokubutsubōeki*, 33, 193–199 (1979) [In Japanese].
4) Kawabe, S. & McLean, D. L.: Electronically recorded waveforms associated with sali-

vation and ingestion behavior of the aster leafhopper, *Macrosteles fascifrons* Stål (Homoptera: Cicadellidae). *Appl. Entomol. Zool.*, 13, 143–148 (1978).

5) Kawabe, S. & McLean, D. L.: Electronic measurement of probing activities of the green leafhopper of rice. *Entomol. Exp. & Appl.*, 27, 77–82 (1980).

6) Kawabe, S., Fukumorita, T. & Chino, M.: Stylectomy of plant-sucking insects using a YAG laser to collect rice phloem sap. *IRRN*, 5(5), 19–20 (1980).

7) Kawabe, S., Fukumorita, T. & Chino, M.: Collection of rice phloem sap from stylets of homopterous insects severed by YAG laser. *Plant & Cell Physiol.*, 21, 1319–1327 (1980).

8) Kawabe, S. et al.: An improved electronic measurement system for studying ingestion and salivation activities of leafhoppers. *Ann. Entomol. Soc. Amer.*, 74, 222–225 (1981).

9) Kishino, K. & Ando, Y.: Insect resistance of the rice plant to the green rice leafhopper, *Nephotettix cincticeps* Uhler. 1. Laboratory technique for testing the antibiosis. *Jpn. J. appl. Entomol. Zool.*, 22, 169–177 (1978) [In Japanese with English summary].

10) Kishino, K. & Ando, Y.: Resistance of rice plant to the green rice leafhopper, *Nephotettix cincticeps* Uhler. 2. Fluctuation of antibiosis with the growing stages of the resistant rice varieties. *Jpn. J. appl. Entomol. Zool.*, 23, 129–133 (1979) [In Japanese with English summary].

11) Kogan, M. & Ortman, E. F.: Antixenosis—a new term proposed to define Painter's "nonpreference" modality of resistance. *Bull. Entomol. Soc. Am.*, 24, 175–176 (1978).

12) Koshihara, T.: Resistance of rice varieties against the green rice leafhopper, *Nephotettix cincticeps* Uhler. *Trop. Agr. Res. Ser.*, 5, 221–225 (1971).

13) McLean, D. L. & Kinsey, M. G.: A technique for electronically recording aphid feeding and salivation. *Nature*, **202**, 1358–1359 (1964).

14) McLean, D. L. & Kinsey, M. G.: Identification of electronically recorded curve patterns associated with aphid salivation and ingestion. *Nature*, **205**, 1130–1131 (1965).

15) McLean, D. L. & Kinsey, M. G.: Probing behavior of the pea aphid, *Acyrthosiphon pisum*. I. Definitive correlation of electronically recorded waveforms with aphid probing activities. *Ann. Entomol. Soc. Amer.*, **60**, 400–406 (1967).

16) McLean, D. L.: An electrical measurement system for studying aphid probing behavior. *In* Aphids as Virus Vectors. ed. Harris, K. F. & Maramorosch, K., Academic Press, New York, 277–290 (1977).

(Received for publication, November 7, 1984)