Association of Leafhopper Feeding Behavior with Transmission of Rice Tungro to Susceptible and Resistant Rice Cultivars

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ABSTRACT

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To evaluate selected rice cultivars for their resistance to tungro disease and to the leafhopper *Nephotettix virescens*, a vector of tungro, the feeding behavior of tungro-viruliferous leafhoppers on rice seedlings during an inoculation access was monitored by honeydew tests and electronic recording. Tested seedlings were indexed by enzyme-linked immunosorbent assay for the presence of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). The ninhydrin test monitored leafhopper excretion of ninhydrin-reactive honeydew, indicating leafhopper feeding from the phloem. The safranin test monitored leafhopper excretion of honeydew containing safranin that was absorbed into the xylem elements. The bromocresol-green test monitored leafhopper excretion of basic and acidic honeydew, indicating leafhopper feeding from the phloem and xylem, respectively. The electronic recording monitored waveform patterns, indicating sustained leafhopper feeding from the phloem and xylem. On leafhopper-resistant cultivars, leafhoppers fed mainly from the xylem and, during the feeding, transmitted predominantly RTBV alone. The virus transmission efficiency on the resistant cultivars was high for ASD7, Palasithari 601, and Gam Pai 30-12-15, whereas it was low for ARC 11554 and Ptb 18. On susceptible cultivars IR22 and Taichung Native 1 (TN1), feeding was mainly from the phloem, and transmission was predominantly for both RTBV and RTSV together in high efficiency. On Utri Rajapan and Habiganj DW8, feeding was mainly from the phloem, but virus transmission efficiency was low, indicating their resistance to virus infection. Correlation analysis indicated that differences in the degree of phloem or xylem feedings between virus transmitter and nontransmitter leafhoppers in each cultivar were not significant. In the electronic recording, durations of phloem and xylem feeding of leafhoppers were not significantly different between those that successfully transmitted the viruses on TN1 and those that failed to transmit. There were also no qualitative differences in waveforms between virus transmitter and nontransmitter leafhoppers on each cultivar. Correlations between phloem feeding and virus transmission by leafhoppers and leafhopper survival were significant among cultivars with various resistances to leafhopper. The correlations were not significant within individual leafhoppers in each cultivar. The negative correlation was probably due to variability of leafhoppers in feeding behavior in each cultivar. Nevertheless, resistances of rice cultivars can be differentiated by testing feeding activities of leafhoppers during an inoculation access. The bromocresol-green test was the most appropriate test for this purpose.

Additional keywords: rice green leafhopper, vector resistance.

Tungro (22) is one of the most important virus diseases of rice in the Asian tropics. It is a composite disease caused by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (13,25,27). Both RTBV and RTSV are transmitted efficiently in a semipersistent manner by the green leafhopper (GLH) *Nephotettix virescens* (Distant) (10,14). Rice cultivars resistant to the leafhopper have been grown widely to control tungro infection in the Asian tropics. The resistant cultivars have antibiosis and antixenosis (nonpreference) to the leafhopper and have suffered less feeding damage and tungro infection (4,9,15). Some of the resistant cultivars have succumbed to tungro after a few years of intensive cultivation (15,16,23).

Leafhoppers exposed to plants infected with RTBV and RTSV transmit both viruses, either together or separately (10,11,13,14). Transmission of RTBV by the leafhopper either separately or with RTSV depends on the presence of RTSV (11,13). Generally, on leafhopper-resistant cultivars, fewer leafhoppers transmit the viruses, and transmission is predominantly for RTBV alone (12,15,30). On susceptible cultivars, more leafhoppers transmit the viruses, and the transmission of both RTBV and RTSV is predominant. *Nephotettix* spp. are xylem and phloem feeders on rice (1,18,20,21). Analysis of honeydew (1,9,17–19) and electronic monitoring of feeding activities (17,18,20) indicated that the leafhoppers fed predominantly from the phloem on susceptible cultivars but from the xylem on leafhopper-resistant cultivars. Because of the complex interactions among *N. virescens*, rice, and the tungro-associated viruses, it is difficult to differentiate resistance to the tungro-associated viruses and *N. virescens*.

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To correlate transmission of the tungro-associated viruses and leafhopper feeding activities and to evaluate rice cultivars for their resistance to the viruses, we analyzed and quantified the feeding events of leafhoppers by using three honeydew tests and electronic recording during an inoculation access to seedlings of rice cultivars with various resistances. Preliminary results were reported (5,6).

MATERIALS AND METHODS

Insects and viruses. The colony of *N. virescens* used in this study was collected originally at Laguna, Philippines, and was maintained on a susceptible rice cultivar Taichung Native 1 (TN1) in the greenhouse for several years. Another leafhopper population collected at Koronadal, Southern Philippines, in August 1986 was reared on TN1 plants for five to six overlapping generations before being used for this study. A third leafhopper population, collected at Koronadal in November 1985, was reared on GLH-resistant IR64 for 25 to 26 generations. A tungro isolate originally collected at Laguna, Philippines, has been maintained by successive transfer with leafhoppers on TN1 plants for about 15 yr. Infected TN1 plants showing typical "tungro" symptoms were tested by latex serology (15,24) to select source plants infected with both RTBV and RTSV. Newly emerged leafhopper adults were allowed to feed on source plants for 3 to 4 days. These viruliferous individuals were tested immediately for their feeding activities and virus transmission on test seedlings.

Plants. The rice cultivars used represent four distinct groups that react differently to the leafhopper and tungro infection in greenhouse tests (Table 1). Leafhopper-resistant IR54, moderately

resistant IR36, and TN1 were used to analyze correlation among feeding activities, tungro transmission, and life span. Pregerminated seeds of each cultivar were transferred separately to pots. Seven to 10 days later, seedlings were subjected to feeding-transmission tests, sprayed with an insecticide (monocrotophos 30 EC [Shell Chemicals, Philippines], 25–50 g/L in water), and grown in an insect-proof greenhouse. One month after inoculation, seedlings were indexed by enzyme-linked immunosorbent assay (ELISA). To confirm infectivity, leafhopper adults used in the electronic monitoring were given individually an overnight inoculation access feeding on 7-day-old TN1 seedlings in test tubes. Inoculated seedlings were transplanted to pots and grown in the greenhouse. The seedlings were indexed by ELISA.

ELISA. Antisera to RTBV and RTSV had titers of 1/2,560 and 1/640, respectively, in the ring interface precipitin test (3). One month after inoculation, a leaf tip 10- to 15-cm long was collected from the second youngest leaf of each plant. For a plant of poor growth, the whole plant without the roots was collected. Leaf or plant samples were extracted separately using a leaf and bud press (Erich Pollahne, Wenningsen, FRG) with 0.02 M sodium phosphate buffer (pH 7.4) containing 0.14% NaCl, 0.05% Tween 20, and 0.02% NaN_3. The volume of the buffer solution was adjusted to obtain extracts of 20 times dilution. Extracts were directly tested in ELISA using Immulon II microtiter plates (Dynatech Laboratories, Alexandria, VA). ELISA procedures basically followed those described by Bajet et al (2) and Cabauatan and Hibino (3). For each test sample, one well was used to detect either RTBV or RTSV. On each plate, four wells were reserved for extracts of healthy TN1 leaves, two wells for extracts of TN1 leaves infected with RTBV and RTSV, and four wells for the buffer solution. Samples that gave absorbance at 405 nm that were four times higher than the mean absorbance of healthy control were considered positive for the tested virus.

TABLE 1. Reaction of 10 rice cultivars to tungro disease and the vector leafhopper

Cultivar	Reaction	
	Tungro[y]	Vector[z]
ASD7	S	R
Gam Pai 30-12-15	MR	R
Palasithari 601	S	R
Utri Rajapan	R	S
Habiganj DW8	R	S
TKM6	R	MR–MS
Ptb 18	R	R
ARC 11554	R	R
IR22	S	S
TN1	S	S

[y] Based on percentage infection in the mass inoculation in cages at the International Rice Research Institute: R (resistant) = 0–30%; MR (moderately resistant) = 31–60%; S (susceptible) = 61–100% (27).
[z] Based on seedling bulk damage rating test at the International Rice Research Institute (32); MS = moderately susceptible.

Monitoring of GLH feeding behavior. The feeding activities of tungro-viruliferous leafhoppers during an inoculation access were monitored by analysis of honeydew using bromocresol green (26), ninhydrin, or safranin dye (19), and by an electronic recording device (20). The honeydew methods were tested on 10 cultivars (Table 1) using leafhoppers reared on TN1 plants; the electronic recording was performed on cultivars ARC 11554, Utri Rajapan, and TN1 using the Koronadal population maintained on IR64.

For the bromocresol-green test, an individual tungro-viruliferous leafhopper female was confined in a mylar cage with a test seedling for 1 day. Honeydew excreted by the leafhopper was collected on a bromocresol-green-treated filter paper disk placed around the base of the seedling. Blue color of honeydew spots indicated leafhopper excretion of basic honeydew, and brown or orange color indicated excretion of acidic honeydew. Basic and acidic honeydew collected on each disk was quantified by measuring the area of honeydew spots. Tested seedlings were indexed by ELISA. Twenty seedlings were tested for each cultivar.

For the ninhydrin test, three tungro-viruliferous leafhopper adults were similarly confined in a cage with a test seedling for 7 hr, and honeydew was collected on an untreated filter paper disk. Then, the disk was treated with 0.1% ninhydrin-acetone solution. Bluish or purple honeydew spots indicated leafhopper excretion of honeydew containing amino acids and peptides (19). The area of honeydew spots was measured. Tested seedlings were indexed by ELISA. A total of 40 seedlings was tested in two trials using 120 leafhoppers for each cultivar.

For the safranin test, roots of test seedlings 7–10 days old were dipped in a 0.2% safranin solution for 6–8 hr. All the vascular bundles of the seedlings were stained red with the dye. Three leafhopper adults were confined in a cage for 7 hr with three or four safranin-teated seedlings in a pot. Honeydew was collected on an untreated filter paper disk. Small red honeydew spots indicated leafhopper feeding of safranin solution from the vascular bundles. The number of spots was counted. A total of 20 sets of seedlings was tested for each cultivar. Treated seedlings wilted in 2 or 3 days and therefore were not indexed for the viruses.

Feeding events of tungro-viruliferous leafhopper females on test seedlings were monitored with an electronic recorder at 25 ± 2 C as described by Khan and Saxena (20). A 10-cm-long, 18-μm-diameter gold wire was attached with a silver conducting paint to the dorsum of a 2-hr-starved but water-satiated

TABLE 2. Mean areas of honeydew spots excreted by 20 individual tungo-viruliferous leafhopper adult females that transmitted or failed to transmit either of tungro-associated viruses during a 1-day inoculation access feeding on seedlings of nine cultivars

Resistance to leafhopper[u]	Cultivar	Number of leafhoppers that transmitted viruses(es)	Area of acidic honeydew spots excreted by leafhopper (mm^2)[v]			Area of basic honeydew spots excreted by leafhopper (mm^2)[v]		
			Transmitter[w] (A)	Nontransmitter[x] (B)	Differences between A and B[y]	Transmitter[w] (A′)	Nontransmitter[x] (B′)	Difference between A′ and B′[y]
R	ASD7	9	74 ± 46	78 ± 37	ns	1 ± 0	0	ns
R	Gam Pai 30-12-15	6	54 ± 18	125 ± 107	*	9 ± 9	7 ± 14	ns
R	Palasithari 601	6	47 ± 33	94 ± 90	ns	16 ± 18	8 ± 15	ns
S	Utri Rajapan	4	12 ± 12	32 ± 37	ns	64 ± 33	112 ± 45	ns
S	Habiganj DW8	3	22 ± 6	34 ± 22	ns	115 ± 53	113 ± 58	ns
MR	TKM6	4	207 ± 93	113 ± 119	ns	11 ± 15	3 ± 6	ns
R	ARC 11554	4	74 ± 29	96 ± 72	ns	0	0	...[z]
S	IR22	19	29 ± 28	34	...	110 ± 50	50	...
S	TN1	16	20 ± 13	43 ± 26	ns	99 ± 46	65 ± 36	ns

[u] R = resistant; S = susceptible; MR = moderately resistant.
[v] Area of basic and acidic honeydew spots on bromocresol green-treated filter paper disk was measured.
[w] Average area ± standard deviation of leafhoppers that transmitted either of the viruses.
[x] Average area ± standard deviation of leafhoppers that transmitted neither of the viruses.
[y] * = significant at 5% level; ns = not significant by t-test.
[z] Not analyzed because no observations were made or the number of replications was insufficient.

leafhopper. The other end of the wire was connected to the negative terminal of the recorder. The leafhopper was placed on a leaf blade of a test seedling, and roots of the seedlings were connected to the positive terminal of the recorder through a battery. Voltage signals during a 3-hr inoculation feeding were recorded using a DC chart recorder (Unirecorder Pantos, Nippon Denshi, Japan) at 500-mV amplifier power and at a chart speed of 1.25 cm/min. The leafhopper then was freed from the wire and given an overnight inoculation feeding on a TN1 seedling to confirm its infectivity. Seedlings that were used for monitoring feeding activities and for the overnight inoculation feeding were indexed by ELISA. Duration of characteristic waveforms was measured from the chart. Twenty females were tested individually for each cultivar.

Correlation among feeding activities, tungro transmission, and longevity. Tungro-viruliferous leafhopper adults of the Koronadal population maintained on TN1 were subjected individually to the bromocresol-green test on IR54, IR36, or TN1 seedlings. Then leafhoppers were caged individually on 3-wk-old plants of the respective cultivars to test their survival. Thirty leafhopper females were tested for each cultivar. Areas of acidic and basic honeydew spots, virus transmission, and adult survival on each cultivar were analyzed for possible correlations by *t*-test (8,29).

RESULTS

Relationships based on honeydew tests. In the bromocresol-green test, tungro-viruliferous leafhoppers excreted both basic and acidic honeydew when fed on all cultivars except ASD7 and ARC 11554 (Tables 2 and 3). On ASD7 and ARC 11554, leafhoppers excreted acidic honeydew and little or no basic honeydew. Areas of basic and acidic honeydew spots excreted by individual leafhoppers and their ratio were variable on each cultivar. Leafhoppers excreted more basic but less acidic honeydew on leafhopper-susceptible cultivars (Utri Rajapan, Habiganj DW8, IR22, and TN1) than on leafhopper-resistant cultivars (ASD7, Gam Pai 30-12-15, Palasithari 601, TKM6, and ARC 11554) (Table 2). During the feeding on IR22 and TN1, 80–95% of leafhoppers transmitted both viruses together or separately, and transmission of both RTBV and RTSV was as high as 50–60% (Table 3). During the feeding on Utri Rajapan and Habiganj DW8, however, only 15–20% of leafhoppers transmitted the viruses. During the feeding on the resistant cultivars, 20–45% of leafhoppers transmitted the viruses, and transmission was predominantly (90–100%) for RTBV alone (Table 3). Gam Pai 30-12-15, Palasithari, and TKM6 were infected with RTBV alone. The differences in areas of basic and acidic honeydew spots excreted by virus transmitters and nontransmitters were not significant in all cultivars except in Gam Pai 30-12-15 (Table 2).

In the ninhydrin test, leafhoppers excreted greater ninhydrin-reactive honeydew when fed on the susceptible cultivars than on the resistant cultivars (Table 4). Differences in areas of ninhydrin-reactive honeydew spots excreted by virus transmitters and nontransmitters were not significant in all cultivars except ARC 11554 (Table 4).

In the safranin test, leafhoppers excreted about 40 red honeydew spots in the 7-hr feeding period on ASD7, Palasithari 601, Ptb

TABLE 3. Transmission of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) by tungro-viruliferous leafhopper adult females and their excretion of basic and acidic honeydew during a 1-day inoculation access feeding on seedlings of nine rice cultivars[z]

Cultivar	Viruses	Number of leafhoppers transmitted	Number of leafhoppers that excreted:		
			Basic honeydew	Basic and acidic honeydew	Acidic honeydew
ASD7	RTBV + RTSV	1	0	0	1
	RTBV	8	0	1	7
	RTSV	0	0	0	0
	None	11	0	0	11
Gam Pai 30-12-15	RTBV + RTSV	0	0	0	0
	RTBV	6	0	4	2
	RTSV	0	0	0	0
	None	14	0	6	8
Palasithari 601	RTBV + RTSV	0	0	0	0
	RTBV	5	0	4	1
	RTSV	0	0	0	0
	None	15	0	5	10
Utri Rajapan	RTBV + RTSV	0	0	0	0
	RTBV	4	2	2	0
	RTSV	0	0	0	0
	None	16	0	16	0
Habiganj DW8	RTBV + RTSV	0	0	0	0
	RTBV	3	0	3	0
	RTSV	0	0	0	0
	None	17	0	17	0
TKM6	RTBV + RTSV	0	0	0	0
	RTBV	4	0	2	2
	RTSV	0	0	0	0
	None	16	0	3	13
ARC 11554	RTBV + RTSV	0	0	0	0
	RTBV	2	0	0	2
	RTSV	2	0	0	2
	None	16	0	0	16
IR22	RTBV + RTSV	12	0	12	0
	RTBV	7	0	7	0
	RTSV	0	0	0	0
	None	1	0	1	0
TN1	RTBV + RTSV	8	0	8	0
	RTBV	9	0	9	0
	RTSV	0	0	0	0
	None	3	0	3	0

[z] Honeydew that was excreted during the feeding was collected on filter paper treated with bromocresol green. Blue color of honeydew spots indicates excretion of basic honeydew, and brown or orange color indicates excretion of acidic honeydew.

TABLE 4. Transmission of the tungro-associated viruses and ninhydrin-reactive areas of honeydew spots excreted by three tungro-viruliferous leafhopper adults during a 7-hr inoculation access feeding on each seedling of 10 cultivars

Resistance to leafhopper[u]	Cultivar	Batch of leafhoppers tested (no.)	Batch of leafhoppers that transmitted viruses (no.)	Area of ninhydrin-reactive honeydew spots (mm^2)[v]		
				Virus transmitters[w] (A)	Virus nontransmitters[x] (B)	Difference between A and B[y]
R	ASD7	18	10	62 ± 26	54 ± 29	ns
R	Gam Pai 30-12-15	18	10	117 ± 61	167 ± 65	ns
R	Palasithari	18	8	72 ± 39	49 ± 37	ns
S	Utri Rajapan	18	2	232 ± 68	225 ± 92	ns
S	Habiganj DW8	18	5	120 ± 54	188 ± 115	ns
MR	TKM6	18	9	79 ± 40	89 ± 58	ns
R	ARC 11554	18	3	133 ± 9	64 ± 42	*
R	Ptb 18	18	6	90 ± 37	86 ± 52	ns
S	IR22	18	18	218 ± 106	...[z]	...
S	TN1	18	18	214 ± 180	...	...

[u] R = resistant; S = susceptible; MR = moderately resistant.
[v] Honeydew was collected on filter papers.
[w] Values are average area ± standard deviation over batches of three leafhoppers that transmitted either of the viruses.
[x] Values are average area ± standard deviation over batches of three leafhoppers that transmitted neither of the viruses.
[y] * = significant; ns = not significant at 5% level by *t*-test.
[z] No nontransmitters.

TABLE 5. Number of red honeydew spots excreted by three leafhopper adults caged for 7 hr on three safranin-treated seedlings of nine cultivars[x]

Cultivar	Number of red honeydew spots[y]
ASD7	44 a[z]
Palasithari 601	41 a
Utri Rajapan	23 c
Habiganj DW8	21 c
TKM6	28 bc
Ptb 18	38 ab
ARC 11554	39 ab
IR22	21 c
TN1	22 c

[x] Ten-day-old seedlings were dipped in 0.1% safranin solution for about 6 hr.
[y] Honeydew was collected on filter papers. Average of 20 replications.
[z] In a row, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test.

18, and ARC 11554 compared with about 20 spots on Utri Rajapan, Habiganj DW8, IR22, and TN1 (Table 5). Leafhopper feeding on safranin-treated seedlings looked normal.

Relationships based on electronically recorded waveforms. Split charts in Figure 1 show typical waveforms recorded when leafhoppers fed on seedlings of ARC 11554, Utri Rajapan, or TN1. Arbitrarily, four types of waveform patterns (designated Xi, Pi, P, and S in Fig. 1) were observed in each chart. Xi and Pi were nearly flat patterns in high and low current, respectively, showing a succession of very low amplitude pulse. P and S appeared to be several high and low current peaks in rapid succession, respectively. Relative durations of Xi, Pi, and S, and number of P during the feeding period were variable among leafhoppers that fed on seedlings of each cultivar (Table 6). When leafhoppers fed on ARC 11554, Xi was recorded frequently, and the pattern continued for a longer time than the other patterns (Table 6). When leafhoppers fed on Utri Rajapan and TN1, Pi was recorded frequently, and the pattern continued for a longer time (Table 6).

On ARC 11554, none of the 20 leafhoppers tested transmitted the viruses during the 3-hr feeding on electronic recorder. On Utri Rajapan, one out of 20 leafhoppers transmitted RTBV alone during the 3-hr feeding. On TN1, eight out of 20 leafhoppers transmitted either both RTBV and RTSV or RTBV alone. Overnight inoculation feeding of leafhoppers after the electronic recording revealed that 75–85% of the leafhoppers were infective of the viruses either together or separately. No qualitative differences were observed in the waveforms produced by the virus transmitter and nontransmitter leafhoppers during the feeding on TN1. During the feeding on TN1, virus transmitters recorded longer Pi and shorter Xi than did nontransmitters, although the differences were not significant.

Correlation among feeding mode, tungro transmission, and longevity. Tungro-viruliferous leafhoppers transmitted RTBV and/or RTSV efficiently on TN1 (susceptible), less efficiently on IR36 (moderately resistant to leafhoppers), and even less efficiently on IR54 (resistant to leafhoppers) (Fig. 2A). Transmission of the viruses by leafhoppers was 70% for both RTBV and RTSV on TN1 and 70% for RTBV alone on IR54 (Fig. 2A). During the inoculation feeding, leafhoppers excreted more basic honeydew when fed on TN1 than on IR36 and IR54 (Fig. 2B). The leafhoppers excreted more acidic honeydew on IR36 and IR54 than on TN1. Generally, leafhoppers survived longer on TN1 and IR36 than on IR54 (Fig. 2C). Leafhopper mortality on IR54 was 50% by day 5. This was observed by day 11 on IR36 and TN1.

Scatter diagrams of areas of basic and acidic honeydew spots by virus transmitters and nontransmitters did not indicate that virus transmitters excreted more basic honeydew than did nontransmitters. The area of basic honeydew spots was highly variable on TN1, less variable on IR36, and least variable on IR54. On the other hand, the area of acidic honeydew spots was highly variable on IR54, less on IR36, and least on TN1.

On IR54, the area of basic honeydew spots excreted by leafhoppers and their survival were significantly correlated. However, on the other cultivars, GLH survival and area of acidic or basic honeydew spots were not significantly correlated. The differences in area of acidic or basic honeydew spots or longevity between virus transmitter and nontransmitter on each cultivar also were not significant.

DISCUSSION

In these experiments, we attempted to quantify feeding activities of individual leafhoppers during an inoculation access to seedlings of rice cultivars with various resistances. *Nephotettix* spp. are phloem and xylem feeders on rice plants (1,9,17–21). Feeding sites of the leafhoppers are determined by tracing leafhopper stylet sheaths when they are feeding on plants (17,18,21) or by analysis of honeydew (1,9,17,18). The leafhoppers excrete basic honeydew containing sugars and amino acids during sustained feeding from the phloem but excrete acidic honeydew containing little or none of them during sustained feeding from the xylem (1,9,17,18,20). The leafhoppers feed mainly from the xylem when fed on leafhopper-resistant cultivars but feed mainly from the phloem when fed on susceptible cultivars (1,9,17–20).

In the bromocresol-green test in these experiments, *N. virescens* excreted greater acidic honeydew when fed on leafhopper-resistant

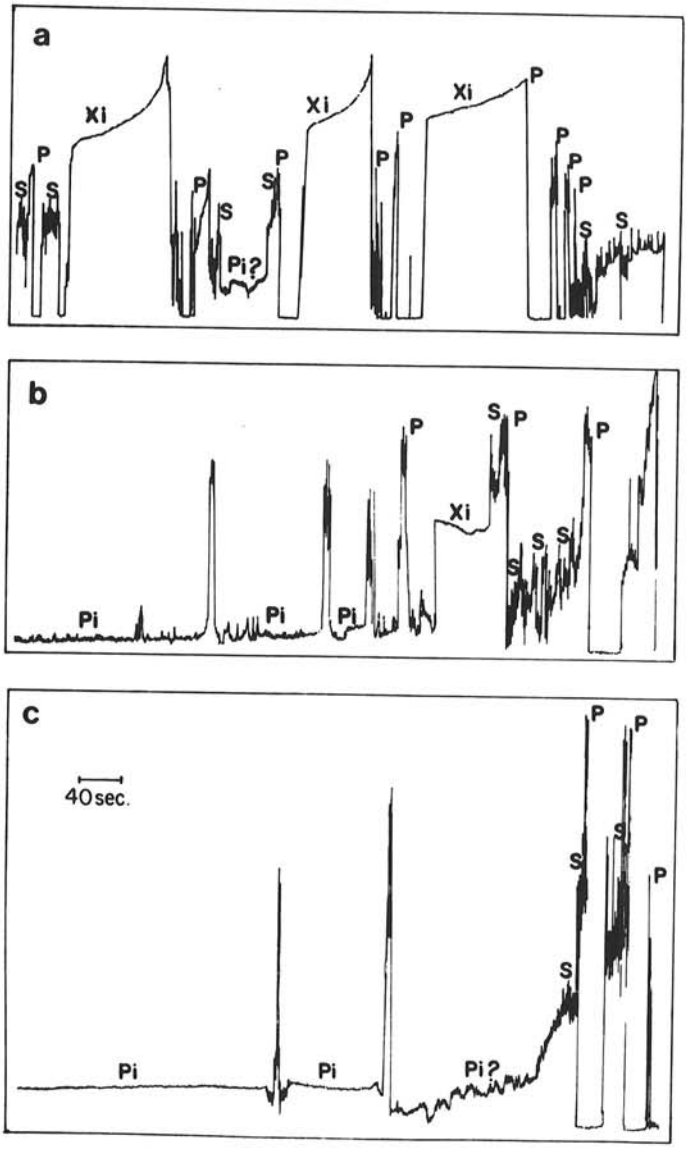

Fig. 1. Section of strip chart showing waveforms produced by a tungro-viruliferous leafhopper female adult during a 3-hr inoculation feeding on seedlings of: **A,** ARC 11554, **B,** Utri Rajapan, or **C,** TN1. Charts are to be read from right to left; P, S, Xi, and Pi are waveform patterns observed during the feeding.

cultivars but greater basic honeydew when fed on leafhopper-susceptible cultivars. In the ninhydrin test, leafhoppers excreted more ninhydrin-reactive honeydew when fed on the susceptible cultivars than on the resistant cultivars. Based on these observations and the previous findings on feeding of *Nephotettix* spp. (1,9,17–20), excretion of basic honeydew by *N. virescens* in the bromocresol-green test was assumed to indicate phloem feeding and excretion of acidic honeydew was assumed to indicate xylem feeding. Also, leafhopper excretion of ninhydrin-reactive honeydew was assumed to indicate phloem feeding. In the safranin test, leafhoppers excreted more red honeydew spots during the feeding on the resistant cultivars than during the feeding on the susceptible cultivars. The excretion of red honeydew spots is likely to indicate xylem feeding (19).

In the electronic recording, two types of waveforms (Pi and Xi in Fig. 1) appeared to indicate sustained leafhopper feedings. Pi continued for a longer time when leafhoppers fed on the susceptible cultivars than when leafhoppers fed on the resistant cultivars, and Xi continued for a longer time when leafhoppers fed on the resistant cultivars. Based on the results obtained in the bromocresol-green and ninhydrin tests, we assumed that Pi indicated phloem feeding and that Xi indicated xylem feeding, although we had no direct evidence to locate the feeding sites. Waveform patterns similar to Pi and Xi have been recorded while *N. virescens* have fed on rice plants and have been interpreted as phloem and xylem feedings, respectively (20).

In these experiments, the bromocresol-green test and the electronic recording were able to differentiate the phloem and xylem feedings of individual leafhoppers. In the bromocresol-green test, however, the degree of phloem feeding on the resistant cultivars appeared to be less than that obtained in the ninhydrin test and the electronic recording. Relative degree of xylem feeding on the test cultivars was about comparable to those obtained in the safranin test and the electronic recording. Compared with the bromocresol-green test, the electronic recording seemed to be more reliable in monitoring phloem and xylem feedings of *N. virescens*. However, a single recorder can monitor feeding by only one or two leafhoppers at a time. On the other hand, the bromocresol-green test can monitor feeding of a large number of leafhoppers. The bromocresol-green test is considered appropriate for a routine test involving evaluation of a large number of cultivars.

In the honeydew tests and the electronic recording, feeding of tungro-viruliferous leafhoppers on the leafhopper-resistant cultivars was mainly from the xylem, and transmission of the leafhoppers during the feeding was predominantly for RTBV alone. On the leafhopper-susceptible cultivars IR22 and TN1, the feeding was mainly from the phloem, and transmission was predominantly for both RTBV and RTSV. On other susceptible

TABLE 6. Average duration or number of electronically recorded waveform patterns of tungro-viruliferous leafhopper adult females that transmitted or failed to transmit the tungro-associated viruses during a 3-hr inoculation feeding on seedlings of three rice cultivars [t]

Resistance to leafhopper[u]	Cultivar	Leafhoppers	Number	Number or duration of waveform patterns[v]			
				P (no.)	S (min)	Pi (min)	Xi (min)
R	ARC 11554	Noninfectious[w]	3	21 (15–28)[x]	28 (20–39)	51 (41–57)	85 (50–104)
		Transmitters[y]	0	...	...	...	...
		Nontransmitters[z]	17	32 (4–76)	34 (7–86)	35 (4–74)	96 (46–129)
S	Utri Rajapan	Noninfectious	3	11 (6–14)	27 (20–40)	117 (113–125)	31 (21–44)
		Transmitters	1	3	5	173	2
		Nontransmitters	16	27 (9–57)	36 (9–68)	94 (34–159)	31 (3–83)
S	TN1	Noninfectious	5	10 (5–19)	28 (13–50)	101 (68–170)	37 (0–72)
		Transmitters	8	11 (3–26)	24 (8–43)	120 (55–153)	25 (0–60)
		Nontransmitters	7	12 (1–24)	19 (2–29)	110 (71–165)	43 (7–77)

[t] After the feeding, leafhoppers individually were given an overnight inoculation access feeding on TN1 seedlings.
[u] R = resistant; S = susceptible.
[v] Refer to Figure 1 and text for the waveforms.
[w] Leafhoppers that did not transmit either of the viruses in both overnight and 3-hr inoculation access feedings.
[x] Numbers in parentheses indicate range.
[y] Leafhoppers that transmitted either of the viruses both in the overnight and 3-hr inoculation feedings.
[z] Leafhoppers that transmitted either of the viruses in the overnight feeding but not in the 3-hr inoculation feeding.

cultivars (Habiganj DW8 and Utri Rajapan), transmission was predominantly for RTBV alone, although the feeding was mainly from the phloem. The predominant phloem feeding and low percentage of infection in Habiganj DW8 and Utri Rajapan indicate their resistance to virus infection. In our recent investigations, Habiganj DW8, Utri Rajapan, TKM6, and ARC 11554 showed characteristic resistance to RTSV infection (H. Hibino and R. Daquioag, *unpublished data*). The predominant RTBV transmission by tungro-viruliferous leafhoppers on leafhopper-resistant cultivars may result from predominant feeding from the xylem. Both RTBV and RTSV are reported to be phloem restricted (7,27).

If the two virus-resistant cultivars Habiganj DW8 and Utri Rajapan were not considered, correlations between degree of phloem feeding by leafhoppers and percentage leafhopper transmission of the viruses (RTBV and/or RTSV) or co-transmission of the viruses were significant among cultivars in all tests. These results indicate that greater phloem feeding of the leafhopper generally increases the probability of virus transmission, especially co-transmission, in virus-susceptible cultivars. However, in most cultivars in the honeydew tests, there were no significant differences between virus transmitters and non-transmitters in the degree of phloem and xylem feeding during the inoculation access. In the electronic recording, differences between virus transmitters and nontransmitters in feeding duration from the phloem and xylem were not significant. Correlation between the degree of phloem feeding of leafhoppers and their longevity was significant when analyzed among cuitivars tested, but it was not significant when analyzed within leafhopper individuals. Probably, these results simply reflect variability of leafhoppers used in these experiments. Intrapopulation variability of leafhoppers in feeding behavior was high in each cultivar, although leafhopper populations used have been maintained on the same cultivar for many generations. The observations in these experiments support the results from the barley yellow dwarf-aphid vector system which indicated that, although the amounts of phloem feeding increases the probability of virus transmission, it does not ensure transmission (28).

Because many leafhopper-resistant cultivars were not long lasting (15,16,23), use of cultivars resistant to either of the tungro-associated viruses is desired. Screening rice cultivars for resistance to tungro has been done under natural conditions or by artificial inoculation of seedlings using tungro-viruliferous leafhoppers. Low tungro incidence in cultivars in the screening may be due to their resistance to the leafhopper (9,12,15,21). A simple method that differentiates virus and leafhopper resistance is needed for effective screening of cultivars for resistance to viruses. In all previous reports, cultivars were separately evaluated for their resistance to tungro and the leafhopper (9,12,15,21), and it was difficult to determine whether leafhopper-resistant cultivars also had resistance to the viruses. Cultivars can be evaluated more effectively by testing leafhopper feeding activities during an inoculation access. In these experiments, the bromocresol-green test using tungro-viruliferous leafhoppers combined with ELISA indexing was found to be effective for this purpose.

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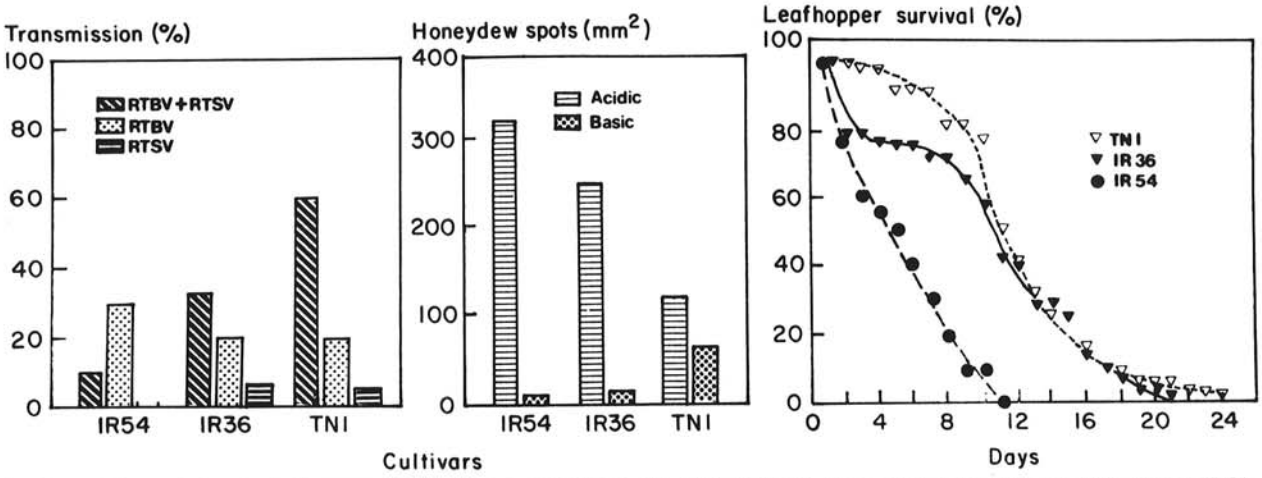

Fig. 2. A, Percent transmission by leafhoppers of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) on IR54, IR36, and TN1 seedlings during a 22-hr inoculation access feeding. **B,** Area of acidic and basic honeydew spots excreted by the leafhoppers during the feeding. **C,** Survival of the leafhoppers on three respective cultivars.

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