Transmission of Two Rice Tungro-Associated Viruses and Rice Waika Virus from Doubly or Singly Infected Source Plants by Leafhopper Vectors

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ABSTRACT

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Transmission efficiencies of leafhopper vectoring rice tungro bacilliform virus (RTBV), rice tungro spherical virus (RTSV), and rice waika virus (RWV), a virus identical or related closely to RTSV, were tested under laboratory conditions. *Nephotettix virescens* efficiently transmitted these three viruses in certain combinations from rice plants infected with both RTBV and RTSV or RWV and also from plants infected with RTSV or RWV alone. *N. cincticeps* also transmitted them but less efficiently than did *N. virescens*. *N. nigropictus* transmitted the three viruses at very low efficiency from plants infected with both RTBV and RTSV or RWV, whereas it transmitted RTSV and RWV rather efficiently from source plants with RTSV or RWV alone. The three leafhoppers were not able to transmit RTBV from plants infected with RTBV alone. *Recilia dorsalis* failed to transmit the three viruses. Possible implications from this study as to how RWV got established and caused an epidemic in Kyushu, Japan, are discussed.

Because polyhedral virus particles were reported to be an infectious entity responsible for tungro disease of rice in 1968 (3), the virus particles were called rice tungro virus. Recent etiological studies indicated, however, that tungro disease was associated with small bacilliform and isometric virus particles (1,5,13,15,16) recently described as rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), respectively (12,14). RTSV isolated from tungro-diseased plants caused no symptoms or slight stunting of rice plants (5,6). So-called tungro symptoms were caused by RTBV, and RTSV intensified the symptoms caused by RTBV infection. Tungro etiology has to be reestablished.

Both RTVB and RTSV were predominantly transmitted by *Nephotettix virescens* Distant in a semipersistent manner (7). RTSV is transmitted alone, but RTBV is dependent on RTSV for its transmission by the leafhopper vector (5,6). *N. nigropictus* Stal., *N. malayanus* Ishihara & Kawase, *N. parvus* Ishihara & Kawase, and *Recilia dorsalis* Motschulsky are also responsible for spread of rice tungro (10,11), although *N. parvus* and *N. malayanus* have little biological relationship with rice plants. In our previous investigation, however, *N. nigropictus* did not transmit RTBV and neither did *R. dorsalis* transmit RTBV or RTSV (7). Rice waika virus (RWV) in Japan is similar in properties (2,13) and serologically homologous to RTSV (6,13,15) and is naturally transmitted by *N. cincticeps* Uhler. *N. cincticeps* is one of the important rice pests in East Asia but its ability to transmit the RTBV/RTSV complex remains unknown. It is also unknown how RTBV is transmitted in combination with RWV.

In this study, RTBV, RTSV, and RWV were tested for transmission together or separately by leafhopper vectors to obtain information on tungro and waika epidemiology. Preliminary results were reported previously (4).

MATERIALS AND METHODS

A tungro-diseased plant was kindly supplied by K. C. Ling, International Rice Research Institute, Philippines, in 1977 and the tungro isolate was

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maintained in rice, *Oryza sativa* L. 'Taichung Native 1' (TN-1), seedlings by successive transfers using *N. virescens*. RTSV was isolated from a tungro-diseased plant (5) and maintained similarly. The RTSV caused no clear symptoms in TN-1 plants. RTBV was isolated from tungro-diseased plants in each experiment by selecting plants containing only RTBV (5). Symptoms caused by RTBV alone included moderate stunting, orange-yellow leaf discoloration, and decreased number of tillers. RWV was collected at Fukuoka, Japan, in 1973 and maintained in TN-1 seedlings by successive transfers using *N. virescens* or *N. cincticeps*.

Virus-free colonies of *N. virescens* and *N. nigropictus* were kindly supplied by J. Hirao and H. Inoue, Kyushu National Agricultural Experimental Station, Japan, and virus-free colonies of *N. cincticeps* and *R. dorsalis* were supplied by I. Kimura, Institute for Plant Virus Research, Japan, and J. Mitsuhashi, National Institute for Agricultural Sciences, Japan, respectively. Each colony was reared on rice seedlings of cultivar Koshihikari in a plastic cage. Acquisition and inoculation accesses were performed at room temperature (24–28 C) under constant illumination by fluorescent lamp. Unless otherwise specified, two male adult leafhoppers given an acquisition access period for 1 day on a virus source plant were allowed an inoculation access period of 8 hr on a TN-1 seedling at the one- or two-leaf stage in a test tube. Each inoculated seedling was tested for virus particles by the leaf-dip method 1 mo after inoculation (5). A leaf tip about 5 cm long was collected from each plant. The tip of a leaf was cut off with a razor blade and the freshly exposed edge was immediately dipped for 1–2 sec into a small drop of 1% neutralized phosphotungstate on a grid covered with a collodion membrane. The edge was cut off again and the new edge was dipped into the same drop. Excess stain was removed with filter paper and the grid was observed under a Hitachi H-500 electron microscope.

Leafhoppers not exposed to rice plants were used as controls in each transmission experiment. None of the inoculated plants developed symptoms typical of virus infection.

RESULTS

Infection of rice plants in sequential combinations with RTBV and RWV. A virus-free colony of *N. virescens* was given sequential acquisition accesses to RWV for 1 day and then to RTBV for 8 hr. As a control, leafhoppers were given access periods alternatively first to RTBV and then to RWV. Leafhoppers given access to RWV first and then RTBV transmitted both RTBV and RWV or RTBV alone. Leafhoppers given access to RTBV first and then RWV failed to transmit RTBV but did transmit RWV (Table 1). Symptoms on rice plants containing both RTBV and RWV included stunting, yellow-orange leaf discoloration, and interveinal chlorosis similar to the symptoms on rice plants infected with both RTBV and RTSV (5). These plants were used as inoculum sources in the following transmission tests.

Transmission by *N. virescens* and *N. cincticeps*. Virus-free colonies of *N. virescens* and *N. cincticeps* were given acquisition access simultaneously on a plant infected with RTBV plus RTSV or RWV or with each separate virus. *N. virescens* and *N. cincticeps* were given separate inoculation accesses to compare their ability to transmit the three viruses. *N. virescens* transmitted the three viruses in high efficiencies from all source plants tested except one infected with RTBV alone. *N. cincticeps* also transmitted the three viruses, although transmission efficiencies were lower than those of *N. virescens* (Table 2). *N. cincticeps* also failed to transmit RTBV from a source plant infected with RTBV alone.

Transmission by *N. nigropictus*. Virus-free colonies of *N. nigropictus* and *N. virescens* were given acquisition access simultaneously on a plant infected with RTBV and/or RTSV or RWV. None of the *N. nigropictus* exposed to both RTBV and RTSV or RWV transmitted both viruses together, but a few leafhoppers transmitted RTBV or RTSV alone (Table 3). *N. nigropictus* exposed to either RTSV or RWV alone, however, transmitted them efficiently. In rough estimation, amounts of RTSV or RWV revealed by electron microscopy of leaf-dip preparations were similar in leaves infected with RTSV or RWV alone and in leaves infected with both RTBV and RTSV or RWV. Therefore, differences in transmission efficiencies apparently were not caused by differences in amounts of viruses in singly and doubly infected plants. On the other hand, *N. virescens* transmitted the three viruses efficiently from the same source plants. In other tests, five *N. nigropictus* exposed to both RTBV and RTSV were allowed an inoculation access on individual rice seedlings. The transmission tests were repeated five times using 950 leafhoppers. Symptoms appeared on eight of 190 seedlings inoculated. All eight seedlings contained RTBV alone. Ninety-five seedlings were inoculated similarly using 475 leafhoppers exposed to both RTBV and RWV. Five seedlings developed symptoms and contained RTBV alone.

Transmission by *R. dorsalis*. Five *R. dorsalis* fed on each source plant for 2 days were allowed an inoculation access period of 8 hr on a rice seedling. Transmission tests were repeated three times using 770 leafhoppers for recovery from a mixture of RTBV and RTSV. Tests were also done using 150 leafhoppers

Table 1. Effects of rice waika virus (RWV) on the transmission of rice tungro bacilliform virus (RTBV) after sequential acquisition accesses by *Nephotettix virescens*

Acquisition access		Virus transmission[a] (no. plants with virus)			
1st for 1 day	2nd for 8 hr	RTBV + RWV	RTBV	RWV	None
RWV	···	0	0	11	12
···	RWV	0	0	5	15
RWV	RTBV	5	9	0	10
RTBV	RWV	0	0	18	14

[a]Two leafhoppers were allowed an inoculation access period of 8 hr on a rice seedling (cultivar Taichung Native 1).

Table 2. Transmission of rice tungro bacilliform virus (RTBV), rice tungro spherical virus (RTSV), and rice waika virus (RWV) from virus source plants containing RTBV and/or RTSV or RWV by leafhopper vectors *Nephotettix virescens* and *N. cincticeps*

Inoculum source containing:	Vectors	Virus transmission[a] (no. plants with virus): RTBV + RTSV (or RTBV + RWV)	RTBV	RTSV (or RWV)	None
RTBV + RTSV	*N. virescens*	29	6	2	5
	N. cincticeps	2	5	5	37
RTBV	*N. virescens*	0	0	0	21
	N. cincticeps	0	0	0	22
RTSV	*N. virescens*	0	0	25	3
	N. cincticeps	0	0	8	12
RTBV + RWV	*N. virescens*	11[b]	21	0[c]	9
	N. cincticeps	1[b]	10	1[c]	40
RWV	*N. virescens*	0[b]	0	21[c]	4
	N. cincticeps	0[b]	0	7[c]	16

[a]Two leafhoppers exposed to a source plant for 1 day were allowed subsequently an inoculation access period of 8 hr on a rice seedling (cultivar Taichung Native 1).
[b]No. plants with RTBV + RWV.
[c]No. plants with RWV.

Table 3. Transmission of rice tungro bacilliform virus (RTBV), rice tungro spherical virus (RTSV), and rice waika virus (RWV) from virus source plants containing RTBV, and/or RTSV or RWV by leafhopper vectors *Nephotettix virescens* and *N. nigropictus*

Inoculum source containing:	Vectors	Virus transmission[a] (no. plants with virus) RTBV + RTSV (or RTBV + RWV)	RTBV	RTSV (or RWV)	None
RTBV + RTSV	*N. virescens*	23	7	4	16
	N. nigropictus	0	1	1	58
RTBV	*N. virescens*	0	0	0	25
	N. nigropictus	0	0	0	24
RTSV	*N. virescens*	0	0	21	0
	N. nigropictus	0	0	6	28
RTSV + RWV	*N. virescens*	7[b]	8	1[c]	6
	N. nigropictus	0[b]	1	0[c]	34
RWV	*N. virescens*	0[b]	0	17[c]	3
	N. nigropictus	0[b]	0	5[c]	31

[a] Two leafhoppers exposed to a virus source plant for 1 day were allowed subsequently an inoculation access period of 8 hr on a rice seedling (cultivar Taichung Native 1).
[b] No. plants with RTBV + RWV.
[c] No. plants with RWV.

Table 4. Transmission efficiencies of rice tungro bacilliform virus (RTBV), rice tungro spherical virus (RTSV), and rice waika virus (RWV) from rice plants infected with RTBV and/or RTSV or RWV by leafhopper vectors[a]

Leafhopper vector	Efficiency of transmission from source plants with RTBV + RTSV	RTBV + RWV	RTBV	RTSV	RWV
Nephotettix virescens	+++[b]	+++	−	+++	+++
N. cincticeps	++	++	−	++	++
N. nigropictus	+	+	−	++	++
Recilia dorsalis	−	−	−	−	−

[a] Efficiencies based on data in Tables 1–3 and text.
[b] +++ = Transmission with high efficiency (>70%), ++ = transmission with intermediate efficiency

for a mixture of RTBV and RWV, 150 for RTBV alone, 190 for RTSV alone, and 180 for RWV alone. None of the inoculated seedlings developed symptoms nor did they contain virus particles.

DISCUSSION

Results of transmission tests are summarized in Table 4. *N. virescens, N. cincticeps*, and *N. nigropictus* transmitted RTBV and RTSV plus RWV, and their transmission efficiencies decreased in due order. This is the first report on transmission of the rice tungro complex by *N. cincticeps*.

N. nigropictus transmitted either RTBV or RTSV with very low efficiency from plants infected with both viruses. Negative transmission of RTBV by *N. nigropictus* in the previous investigation (7) might have been due to an insufficient number of leafhoppers tested or a possibly lower transmission efficiency of the colony. It is known that the percentage of active transmitters of the tungro complex varies from 0 to 45% in different colonies of *N. nigropictus* (8,9,17). Hino et al (8) reported that the symptoms after transmission by *N. nigropictus* were milder and recoveries from some of those plants using *N. virescens* were unsuccessful. Those plants presumably were infected with RTBV alone.

It is known that 1–8% of *R. dorsalis* are able to transmit the tungro complex (8,10). In this experiment, none of *R. dorsalis* tested transmitted RTBV, RTSV, or RWV. Colonies used in this and other (7) experiments may not be able to transmit any of the three viruses. RWV has not been transmitted by *R. dorsalis* in Japan (2).

In these experiments, RWV assisted in transmission of RTBV as did RTSV (5), and the symptoms that resulted from infection with both RTBV and RWV were similar to those of the tungro complex caused by double infection with RTBV and RTSV. These results substantiate that RWV is identical or closely related to RTSV (13).

RWV was discovered in 1967 in the northern part of Kyushu and later reached an epidemic proportion, but RTBV has never been found anywhere in Japan. Identity or close relationship between RTSV and RWV (13) indicates that RTSV alone was responsible for the epidemic. The primary source of waika infection still remains to be found. The tungro complex is transmitted only by leafhopper vectors. Therefore, RTSV might have been introduced by *N. virescens* to Kyushu from a rice tungro epidemic area. Migrating leafhoppers might carry both RTSV and RTBV because rice plants are generally infected with both viruses in tungro epidemic areas (13,15). Relationships between tungro-associated viruses and vectors shown in these experiments indicate a possible explanation of how RTSV alone could have caused the epidemic in Kyushu. If *N. virescens* carrying both RTBV and RTSV arrived in Kyushu through overseas immigration or by flight from Southeast Asia, where tungro is predominant, the leafhoppers could have transmitted the viruses to several rice plants in small areas because leafhoppers retain the viruses for only 2–3 days (7). *N. cincticeps* is distributed widely with a high population in Japan, and *N. virescens* and *N. nigropictus* are found in Nansei Islands and on rare occasion in the southern district of the Kyushu mainland. Therefore, the viruses might have been transmitted farther from those plants by the native vector *N. cincticeps*. Some *N. cincticeps* that carried both viruses might have transmitted RTBV alone or both viruses or RTSV alone to surrounding rice plants. Plants infected with RTBV would not be a virus source for further transmission by the leafhopper, but plants infected with both RTBV and RTSV should be a good source. Because *N. cincticeps* is an inefficient vector of the RTBV/RTSV complex, the number of plants infected with both RTBV and RTSV might not increase or could disappear in the field, with a low population level of *N. cincticeps*. Plants doubly infected may not be able to overwinter. RTSV could be transmitted rather efficiently by *N. cincticeps* from plants infected with RTSV alone. The RTSV thus spread and established in Kyushu might be RWV; RWV can overwinter in ratoons in Kyushu (9).

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