Serological Monitoring of Rice Tungro Disease Development in the Field: Its Implication in Disease Management

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ABSTRACT

Rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) were monitored in transplanted rice (Oryza sativa) by latex test or by enzyme-linked immunosorbent assay (ELISA). RTSV was detected 1 wk after transplanting, and the incidence increased rapidly within 2-3 wk. Dual infection by RTBV and RTSV was detected 2 wk after transplanting. The infectivity of leafhoppers collected in the field corresponded with the disease development. However, the development of the tungro disease differed in cultivars with vector resistance and in different planting seasons. The main tungro infection occurred after transplanting, based on the results of covering plants with mesh screens, setting up an unprotected nursery in a tungro-affected field, and treating with insecticide at different growth stages of rice.

Tungro (12) is the most important virus disease of rice (Oryza sativa L.) in South and Southeast Asia and is caused by the rice tungro bacilliform virus (RTBV) and the rice tungro spherical virus (RTSV). Generally, RTBV causes the tungro symptoms, including yellow-orange discoloration and plant stunting. RTSV enhances the symptoms caused by RTBV, but alone causes only very mild stunting (6). The tungro viruses are transmitted semipersistently by leafhoppers, notably Neophytettix virescens (Distant). RTSV can be transmitted alone by leafhoppers, but the transmission of RTBV by the vectors is dependent on RTSV (5). Rice cultivars with resistance to N. virescens have been planted widely in the Philippines to manage tungro (11).

Little information is available on tungro infection in nurseries, whereas various studies on the development of tungro disease in paddy fields have been reported (9,10,18,19,21). Those results, however, were based on symptoms and did not consider tungro as caused by RTBV and RTSV. Diagnosis based on serology is essential to understanding the epidemiology of tungro, especially since RTSV does not induce distinct symptoms.

In this work, serological indexing was used to determine the role of nursery in tungro epidemics and the development of RTBV and RTSV infection in the field in relation to the virus infectivity of green leafhoppers. Preliminary results have been published (20).

MATERIALS AND METHODS
Plants. Rice cultivars with differences in vector resistance were selected. Taichung Native 1 (TN1) and IR22 are sus-
ceptible, IR36 and IR42 are moderately resistant, and IR54 and IR58 are highly resistant to *N. virescens* (4,8). The seeds of test cultivars were germinated in running water for 2 days and then sown in wetted nurseries. To protect the seedlings from leaffoppers, some nurseries and field plots were covered with fiber-glass mesh screens. At 21–26 days after sowing, seedlings were transplanted at one seedling per hill in virus development studies and at two to three seedlings per hill in nursery infection studies at 20 × 20 cm spacing in plots either in an experimental field or in an insect-proof greenhouse at the International Rice Research Institute, Laguna, Philippines. The plots were arranged 2 m apart in a randomized complete-block design when several treatments were involved. No insecticide was applied unless stated.

Tungro disease incidence was scored based on symptoms, and RTBV or RTSV incidence was assessed in leaf samples by latex test or enzyme-linked immunosorbent assay (ELISA).

**Leafhopper infectivity and density.** The infectivity of field-collected vectors, *N. virescens* and *Nephotettix nigropictus* (Stål), was tested by confining individual insects in test tubes with 7-day-old TN1 seedling for 1 day. Inoculated seedlings were grown in a greenhouse and indexed by ELISA 3–4 wk after inoculation. The total number of *N. virescens* and *N. nigropictus* collected by 10 sweeps of a 33-cm-diameter insect net was used to determine leafhopper density.

**Serology.** A leaf sample about 10 cm long was taken from the second youngest leaf of a tiller of each plant. The latex test was conducted following the procedure of Omura et al. (17). Latex particles were sensitized with partially purified immunoglobulin (IgG) at 1:150 dilution for RTBV and at 1:120 dilution for RTSV. ELISA was done as described by Bajet et al. (2). The concentration of coating IgG was 1 μg/ml for RTBV and 1.5 μg/ml for RTSV. IgG-alkaline phosphatase conjugate was diluted at 1/2,000 for RTBV and at 1/1,000 for RTSV. The reactions were evaluated at 405 nm in the MicroElisa Minireader MR 590 (Dynatech Laboratories, Virginia). Absorbances more than twice the mean of four healthy control readings were considered positive.

**Virus detection in artificially inoculated plants.** ELISA replaced the latex test in later experiments when it became available. This experiment was conducted to determine the earliest time when the tungro viruses are detectable by latex test and ELISA in 21-day-old (the usual age of seedlings for transplanting in the Philippines) TN1 seedlings inoculated in the greenhouse. Adult *N. virescens* were given a 3-day acquisition access period on TN1 plants infected with both RTBV and RTSV. Immediately afterwards, the insects were transferred to the TN1 seedlings in mylar cages at one or five insect(s) per seedling for a 24-hr inoculation access period. Similarly, TN1 seedlings were inoculated by leafhopper adults that were exposed to TN1 plants infected with RTSV alone. A leaf sample was taken from each plant daily for 7 days, and at 12 and 18 days after inoculation (DAI), and tested by latex test and ELISA. A total of 150 seedlings were inoculated per virus source by one or five leafhopper(s) in 2 trials.

**Virus infection in the nursery.** In March–May 1987, TN1 seedlings from covered and uncovered nurseries were transplanted in 4 × 4 m plots in the field with four replications, which were also uncovered or covered with mesh screens. At 44 days after transplanting, tungro incidence was recorded and leafhopper density was estimated in each plot. For leaf sampling, five subplots were arranged quincuncially in each plot. Sixteen leaf samples (4 × 4 hills) from each subplot were examined by the latex test.

In April–June 1989, germinated TN1 seeds were sown in an uncovered nursery established in the middle of a field where 12-wk-old TN1 plants had 98% tungro infection. Seedlings were later transplanted to four 7.5 × 8 m plots in the greenhouse and four 5.5 × 9 m plots in the field. At 20 and 35 days after transplanting, all plants were scored for symptoms. For ELISA, 25 leaf samples

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**Fig. 1.** Incidence of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) as detected by enzyme-linked immunosorbent assay at different times after inoculating 21-day-old Taichung Native 1 plants 24 hr after inoculation feeding by (A) one leafhopper and (B) five leafhoppers that fed on plants infected with both RTBV and RTSV, or by (C) one leafhopper and (D) five leafhoppers that fed on plants infected with RTSV alone.

**Table 1.** Green leafhopper density and incidence of tungro disease, rice tungro bacilliform virus (RTBV), rice tungro spherical virus (RTSV), and RTBV and RTSV 44 days after Taichung Native 1 plants grown in covered and uncovered nurseries were transplanted to covered and uncovered plots

<table>
<thead>
<tr>
<th>Nursery</th>
<th>Field</th>
<th>Green leafhopper (no.)</th>
<th>Tungro incidence (%)</th>
<th>Virus incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover</td>
<td>Cover</td>
<td>0.0 b</td>
<td>0.0 c</td>
<td>0.0 c</td>
</tr>
<tr>
<td>Cover</td>
<td>No cover</td>
<td>14.8 a</td>
<td>40.1 b</td>
<td>36.6 b</td>
</tr>
<tr>
<td>No cover</td>
<td>Cover</td>
<td>10.6 a</td>
<td>1.4 c</td>
<td>2.9 c</td>
</tr>
<tr>
<td>No cover</td>
<td>No cover</td>
<td>14.6 a</td>
<td>53.1 a</td>
<td>48.0 a</td>
</tr>
</tbody>
</table>

*Indexed by latex test.
*N. virescens* and *N. nigropictus* catches per 10 sweeps.
Based on visual assessment.
Means in the same column followed by same letter are not significantly different according to Duncan's multiple range test (*P* = 0.05).
(5 x 5 hills) were taken from each of 10 subplots arranged in a W pattern (3). Leafhopper density in the field and in the greenhouse 35 days after transplanting was determined.

In March-May 1989, the effects of insecticide treatments on virus infection in the nursery and after transplanting were examined. Cypermethrin (Cymbush, 5 EC) at 25 g a.i./ha and buprofezin (Applad, 25 WP) at 500 g a.i./ha were combined and applied on IR22 plants, either in the nursery 12 days after sowing, in the field 2 or 16 days after transplanting, or in combinations of the three treatments. Untreated plots served as controls. Seedlings were transplanted in 10 x 10 m plots with four replications. At 14, 33, and 61 days after transplanting, 16 leaf samples (4 x 4 hills) were collected from each of 10 subplots arranged in a W pattern and tested by ELISA.

Development of virus infection and vector infectivity after transplanting. In September-November 1987, TN1 seedlings from an uncovered nursery were transplanted to two 20 x 20 m plots. Tungro incidence was scored at weekly intervals. Twenty-five leaf samples (5 x 5 hills) were taken from each of 10 subplots arranged in a W pattern and tested by ELISA. The infectivity of leafhoppers collected by sweep net in the transplanted plots was also determined weekly from 7 to 34 days after transplanting.

In July-September 1988, TN1 seedlings from a covered nursery were transplanted in six 10 x 10 m plots to examine the sequence of RTBV and R TSV infection in marked individual plants. Leaf samples from each of nine plants (3 x 3 hills) in 33 subplots arranged circumcuncially were collected at weekly intervals starting 8 days after transplanting and tested by ELISA.

In the 1985 wet season (August-October), seedlings of TN1, IR22, IR36, IR42, IR54, and IR58 were separately transplanted to 2 x 2 m plots with four replications to determine the effect of vector resistance on the development of virus infection. At weekly intervals starting 30 days after transplanting, leaf samples were collected from 36 plants (inner rows of 6 x 6 hills) in each plot and assessed by latex test. A similar experiment was conducted in the 1986 dry season (January-March), when leaf samples were

Table 2. Percentage incidences of tungro disease, rice tungro bacilliform virus (RTBV), rice tungro spherical virus (RTSV), and RTBV and R TSV in Taichung Native 1 plants from a nursery established in a tungro-affected field 20 and 35 days after transplanting in a greenhouse and in a field

<table>
<thead>
<tr>
<th>Seedlings transplanted</th>
<th>20 Days after transplanting</th>
<th>35 Days after transplanting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tungro incidence&lt;sup&gt;2&lt;/sup&gt;</td>
<td>RTBV and RTSV</td>
</tr>
<tr>
<td>Screenhouse</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Field</td>
<td>27</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>2</sup>Indexed by enzyme-linked immunosorbent assay.
<sup>3</sup>Based on visual assessment.
<sup>4</sup>Hypothesis test for means using t test (P = 0.05). In a column, NS indicates no significant differences.

Fig. 2. Percentage of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) in IR22 plants treated with insecticides in the nursery 12 days after sowing, in the field 2 or 16 days after transplanting, and in combinations of the three treatments. Plants were assayed by enzyme-linked immunosorbent assay at 14, 33, and 61 days after transplanting.
collected at 30, 45, and 60 days after transplanting.

RESULTS

Virus detection in inoculated plants by latex test and ELISA. The tungro viruses were first detected by ELISA 3 DAI in TN1 plants inoculated with both RTBV and RTSV by one or five leafhopper(s) (Fig. 1). Thereafter, the percentage of plants positive for RTBV was higher than for RTSV. At 5 DAI, 92% of the plants inoculated by five leafhoppers were positive by ELISA for RTBV and RTSV. In plants inoculated with RTSV alone, however, the virus was detected by ELISA at 4 DAI with five leafhoppers and at 5 DAI with one. The percentage of RTSV-infected plants remained low until 7 DAI (Fig. 1). Regardless of virus source and number of leafhoppers used in the inoculation, the tungro viruses were detected by the latex test 1 day later than by ELISA. However, at 12 and 18 DAI, the percentages of plants detected with the viruses by ELISA and by latex test were similar (data not shown).

Virus infection in the nursery. In the March–May 1987 trial, seedlings raised in covered or uncovered nurseries and then transplanted into covered field plots developed low incidence of tungro (Table 1). In contrast, seedlings from the covered nursery transplanted to uncovered field plots had high incidences of tungro. Tungro incidence was also high in uncovered plots planted with seedlings from the uncovered nursery. There were no significant differences in leafhopper catches among the treatments, except in plants protected continuously in both nursery and field.

While tungro incidence remained low when seedlings from a nursery located in the middle of a field with severe tungro were transplanted to a screenhouse, tungro increased to a high level in the field transplanted with seedlings from the same nursery. At 35 days after transplanting, tungro disease incidence was only 7% in the screenhouse, whereas it was 99% in the field (Table 2). Leafhopper catches were not very different in the screenhouse (three adults and six nymphs) and in the field (nine adults and seven nymphs) 35 days after transplanting.

Insecticides applied in the nursery did not affect the incidence of tungro after transplanting. At 14 days after transplanting, the percentage of infected plants was generally low and did not vary greatly among treatments (Fig. 2). However, the percentage of RTBV and RTSV infection 61 days after transplanting was low in plants treated 16 days after transplanting, in plants treated in the nursery and 16 days after transplanting, in plants treated 2 and 16 days after transplanting, and in plants treated in the nursery and 2 and 16 days after transplanting. In other treatments, the percentage of plants infected with both RTBV and RTSV increased.

Development of virus infection in the field. In the September–November 1987 trial, RTSV was detected on the first sampling date (8 days after transplanting) (Fig. 3). Typical tungro symptoms were not apparent until the following week, when dual infection of RTBV and RTSV was detected. The percentage of plants infected with RTSV alone was highest about 1 mo after transplanting. Thereafter, detection of RTBV was less, but detection of dual infection increased. Serological detection of RTBV corresponded with the appearance of visual symptoms. The infectivity of the vectors collected in the same field was 8 and 12% for RTSV at 7 and 12 days after transplanting, respectively, and exceeded 60% for both RTBV and RTSV at 21 days after transplanting and thereafter (Fig. 3). The presence of leafhoppers infective for both RTBV and RTSV 21 days after transplanting and thereafter was associated with an increase of plants with dual infection 37 days after transplanting (Fig. 3).

Weekly monitoring of TN1 plants in the July–September 1988 trial revealed the sequence of RTBV and RTSV infection in the field. At 8 days after transplanting, 0.2% of the plants were infected by RTSV alone and 0.05% by both RTBV and RTSV. A slight increase in infection occurred at 15 and 22 days after transplanting. At 29 days after transplanting, 4% of the plants were infected with RTBV alone, 34% with RTSV alone, and 29% with both. Of the 517 plants infected with both RTBV and RTSV 29 days after transplanting, 103 (20%) had been healthy and 21 (4%) had been infected with RTSV alone 22 days after transplanting. Of the 1,443 plants which had both RTBV and RTSV 36 days after transplanting, 332 (23%) had been healthy, 375 (26%) had been infected with RTSV alone, and 418 (29%) had been infected with both RTBV and RTSV 29 days after transplanting. These results indicate that the increase in the percentage of plants with both RTBV and RTSV at 29 and 36 days after transplanting was due mainly to the infection of healthy plants with both RTBV and RTSV and to the infection of RTSV-infected plants with RTBV.

The development of virus infection differed among rice cultivars with varying resistance to the vector N. virescens and among planting seasons. In the 1985 wet season, the percentage of plants infected with RTSV alone 30 days after transplanting was highest in cultivars with no vector resistance and lowest in IR54 and IR58, which had high levels of vector resistance (Fig. 4). The percentage of plants infected with RTSV alone remained high until the end of the test period in moderately resistant IR36 and IR42, but declined to a low level in TN1 because of subsequent infection with RTBV. In the 1986 dry season, the percentage of plants infected with RTSV alone 30 days after transplanting was low even in TN1 and IR22. In the later growth stage, the increase in RTSV infection as well as the increase in dual infection was slow in susceptible TN1.
DISCUSSION

In artificial inoculations of 21-day-old TN1 plants in the greenhouse, positive reactions in ELISA for both RTBV and RTSV were obtained by 3 DAI. At 5 DAI, 92% of the plants gave positive reactions. In plants inoculated with RTBV alone, the most positives were detected at 12 DAI. These results indicated that RSV was detected earlier than plants also infected with RTBV. The data suggest that the time lag between virus infection and maximum detection is 5-6 days in plants doubly infected or in those with RTBV alone, but is about 12 days in plants infected with RSV alone. The latex test detected both tungro viruses only 1 day later than ELISA, indicating that the sensitivity of the two assays was similar.

The role of infection in nurseries in the spread of tungro has been controversial. Control of the disease in nurseries was recommended in some reports (14-16). In contrast, Inoue and Ruay-Aree (10) and van Halteren (21) demonstrated negligible infection in nurseries. Hino et al (9) reported that major tungro infection occurred after transplanting, although a high level of infection occurred in a nursery in one of their experiments where a high population of viruliferous insects occurred. In this study, the trials at different seasons showed that neither covering the nursery with mesh screens, sowing an unprotected nursery in a tungro-affected field, nor applying insecticides in the nursery affected the spread of infection or the vector populations in the field after transplanting. Insect infestation from surrounding fields was precluded in some of the experiments and led to slow disease spread, mainly from the seedlings infected in the nursery and the leafhoppers that developed after transplanting. These results show that virus infection is low during the nursery period of 21-26 days, and that vector control by insecticide at this stage is not an effective management option under similar cultivation practices.

Serology revealed that infection of plants with RSV alone preceded infection with RTBV in fields transplanted with TN1, IR36, and IR42. Because there are no discernable symptoms on plants infected with RSV alone, the disease is not manifest soon after transplanting. After the incidence of plants infected with RSV alone reached a maximum level, about 4 wk after transplanting, it began to decline as subsequent infection of RTBV began. Hibino et al (8) reported predominant RTBV infection in vector-resistant cultivars by natural inoculation, with RTBV- and RSV-infected plants as virus sources. In this study, RSV was more prevalent than RTBV in the moderately resistant cultivars, IR36 and IR42, in the field. These observations can be explained by the independent transmission of RSV (5,7) and the widespread occurrence of RSV in fields where little or no RTBV occurs (1). The early infection of plants with RSV indicates the movement of vectors largely infective with RSV alone soon after transplanting. The development pattern of the tungro viruses in transplanted fields varied depending on cultivar and cropping season. The transition from a single infection of mostly RSV to dual infection was generally slow in the dry season and in fields planted to cultivars with resistance to the vector leafhopper. However, the level of infection in susceptible IR22 in the wet season was unexpectedly lower than that of TN1 for reasons not known.

Major tungro infection occurred after transplanting and was likely initiated by viruliferous insects from surrounding fields. The management of tungro, particularly by insecticide application, should therefore be focused on the first 2 wk after transplanting, a stage of rice growth that is very susceptible to tungro

![Figure 4](image_url)

Fig. 4. Development of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) infection in six rice cultivars during the 1985 wet season (WS) and 1986 dry season (DS) croppings, Laguna, Philippines. Rice cultivars Taichung Native 1 (TN1) and IR22 are susceptible, IR36 and IR42 are moderately resistant, and IR54 and IR58 are highly resistant to the leafhopper vector, *Nephotettix virescens.*
infection (13,14). Using cultivars with high vector resistance and the proper timing of insecticide application would lead to more effective and economical management of rice tungro disease.

LITERATURE CITED