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CRITICAL TIME FOR THE OCCURRENCE AND DEVELOPMENT OF TUNGRO INFECTION IN THE FIELD

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ABSTRACT

Field trials were conducted at IRRI farm to determine the time tungro infection likely occurs and how it spreads in the field.

Tungro infection, at very low rates, occurred in plants from uncovered seedbeds. However, no difference in infection to any of the tungro viruses occurred between the plants from covered and uncovered seedbeds after transplanting in the field. Although no symptoms were discernible, RTSV infection was detected in the plants by latex test at 14 DAT. Tungro symptoms were manifested by the infected plants between 22 and 35 DAT coinciding with the detection of both RTBV and RTSV. Tungro infection on IR62 and IR64 also occurred in the same period of time.

At 37 DAT, no difference in infection in the three distance classes of surrounding (direct neighbor, diagonal, distant) hills was obtained in TN1, IR36, and IR54 plants. With time, more direct neighbor hills of TNI plants were infected while the infection rates in the three distance classes in IR36 and IR54 plants did not differ. Under controlled conditions in field cages, viruliferous leafhoppers spread tungro to rice plants nearer the virus source. Hence, the spread of tungro infection is more likely to occur in plants in proximity to the infected plants of a susceptible variety.

The possible role of RTSV and the seedbeds in tungro epidemiology is discussed.

Introduction

One of the major constraints to rice production in South and Southeast Asia is tungro. It is transmitted by several species of leafhoppers in a semipersistent or transitory manner (Ling and Tiongco, 1979). The most efficient vector species is the rice green leafhopper, *Nephotettix virescens* (Distant) (Ling, 1972).

Based on the new understanding that tungro is a composite disease caused by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (Hibino *et al.*, 1978; Omura *et al.*, 1983), significant findings on tungro transmission (Hibino *et al.*, 1979; Hibino, 1983) and symptomatology (Hibino *et al.*, 1978; Hibino, 1983) improved our knowledge about the disease and in turn gave a clearer picture of tungro epidemiology.

The results of field experiments which indicate the time tungro infection likely occurs and how it spreads in the field is reported.

Materials and Methods

Field experiments

A preliminary trial was conducted from October 1986 to January 1987 to determine the time of occurrence of tungro infection in the field. Seeds of Taichung Native 1 (TN1), a variety susceptible to tungro and the green leafhopper (GLH) were sown on uncovered and covered seedbeds. After 26 days, half of the seedlings from each seedbed were transplanted in a screenhouse while the other half transplanted in the field. The seedlings were spaced at 20 x 20 cms. Tungro infection in the entire plant population was assessed based on symptoms 22, 37, and 65 days after transplanting (DAT). A similar but improved trial was conducted in January to April 1987. The seedlings were sown and apportioned in the same manner as above and transplanted in 5 x 5 m plots in the field laid out in a randomized complete block (RCB) design with four replications. Seedlings transplanted in a screenhouse were planted in 4 x 4 m plots arranged in RCB with two replications. At weekly intervals starting 14 DAT, percentage tungro infection was assessed based on symptoms, and leaf samples were collected and indexed by latex test to determine the tungro-associated viruses in the plants from the field and from the screenhouse. Number of GLH on the plants in the field was recorded weekly starting 21 DAT. Data were taken in 5 sample areas per plot at 16 rice hills per sample area.

Tungro incidence on GLH-resistant varieties was determined using IR62 and IR64. One month after sowing, the seedlings were transplanted with 20 x 20 cm spacing in 10 x 10 m plots laid out in RCB design with four replications. Visual readings of tungro infection in 10 sample areas with 25 rice hills per plot were done at 14, 28, and 37 DAT.

The spatial spread of tungro infection in the field from the initial infected hills to three distance classes of surrounding hills was determined using three varieties with different levels of resistance to GLH. The three distance classes were designated as: a) direct neighbor – rice hill at 20 cm distance parallel or vertical from the initial infected hill, b) diagonal – rice hill at oblique direction approximately 28 cm from the initial infected hill, and c) distant – rice hill other than the first two. TN1 (susceptible), IR36 (moderately resistant), and IR54 (resistant) were transplanted with 20 x 20 cm spacing in 2 x 2 m plots laid out in RCB design with four replications. Visual assessment of the initial infection in the three varieties was recorded at 30 DAT and its spread at weekly intervals thereafter.

The spread of tungro disease was also studied under natural conditions and in field cages measuring 4 x 4 x 1.5 m. One month after sowing on a covered seedbed, TN1 seedlings were transplanted in 4 x 4 m plots and spaced at 20 x 20 cm. The plots were arranged in RCB design with four replications. Four plots were

covered with fiberglass-screen field cages immediately after transplanting. Each plot accommodated 361 plants including one TNI plant infected with both RTBV and RTSV planted at the center of each plot to serve as virus source. Sixteen days after transplanting, each infected plant was covered with a mylar cage and 20 male virus-free *N. virescens* were introduced. After 4 days acquisition access time, the mylar cage was slowly removed to release the insects. After one week, insecticide was applied. Two weeks after insect-release, all plants were scored for symptoms, indexed for infection by the latex test, and hill position of infected plants plotted.

Latex test

Latex particles (Difco Bacto-latex 0.81) were sensitized with partially purified immunoglobulin (IgG) to RTBV or RTSV following the procedure of Omura *et al.* (1984). About 10 cm of the second youngest leaf of each test plant was cut and homogenized separately in 1 ml of 0.05 M Tris-HCl buffer, pH 7.2, using a combined leaf and bud press (Erich Pollahne, Wennigsen, The Federal Republic of Germany). Equal amounts (50 μl) of plant sap and sensitized latex suspension were placed in a small test tube and shaken at 160 oscillations/minute for 30 min. The presence of viruses were indicated by clumping of latex particles observed at 100X magnification using a light microscope.

Results

Seedbed infection

Preliminary trials showed that no tungro infection was observed 22 DAT in all plants from the covered seedbed while infection rates of 0.2% were recorded in plants from the uncovered seedbed planted in the screenhouse and 0.1% in the field. At 37 DAT, the plants in the field from the covered seedbed had 13% disease incidence and those from the uncovered seedbed had 12% which increased to 77 and 79% at 65 DAT. No increase in tungro incidence was observed in plants transplanted in the screenhouse from either seedbeds at 37 DAT (Table 1).

When latex test was used in the improved trial to determine tungro infection, only RTSV infection of 0.02% was obtained 14 DAT in plants from uncovered seedbed transplanted in the screenhouse and none in plants from the covered seedbed. However, plants from both seedbeds transplanted in the field registered 0.94% RTSV infection at 14 DAT and increase to 61% at 35 DAT. Thereafter, a corresponding increase in double infection with RTBV and RTSV was observed as RTSV infection decrease (Fig. 1). Infection with RTBV was low.

An average of 1.1 GLH per plant hill was recorded at different observation time on plants from covered and uncovered seedbeds planted in the field. No difference in the number of GLH per hill was obtained between treatments and over time (Table 2).

Table 1. Percentage tungro infection in Taichung Native 1 rice plants from uncovered and covered seedbeds at different days after transplanting in a screenhouse and in the field

Treatment	*Seedlings transplanted to:*	*% Tungro incidence days after transplanting*		
		22	*37*	*65*
Covered seedbed	screenhouse	0	0	–[a]
	field	0	13	77
Uncovered seedbed	screenhouse	0.2	0.2	–
	field	0.1	12	79

[a]No scoring conducted.

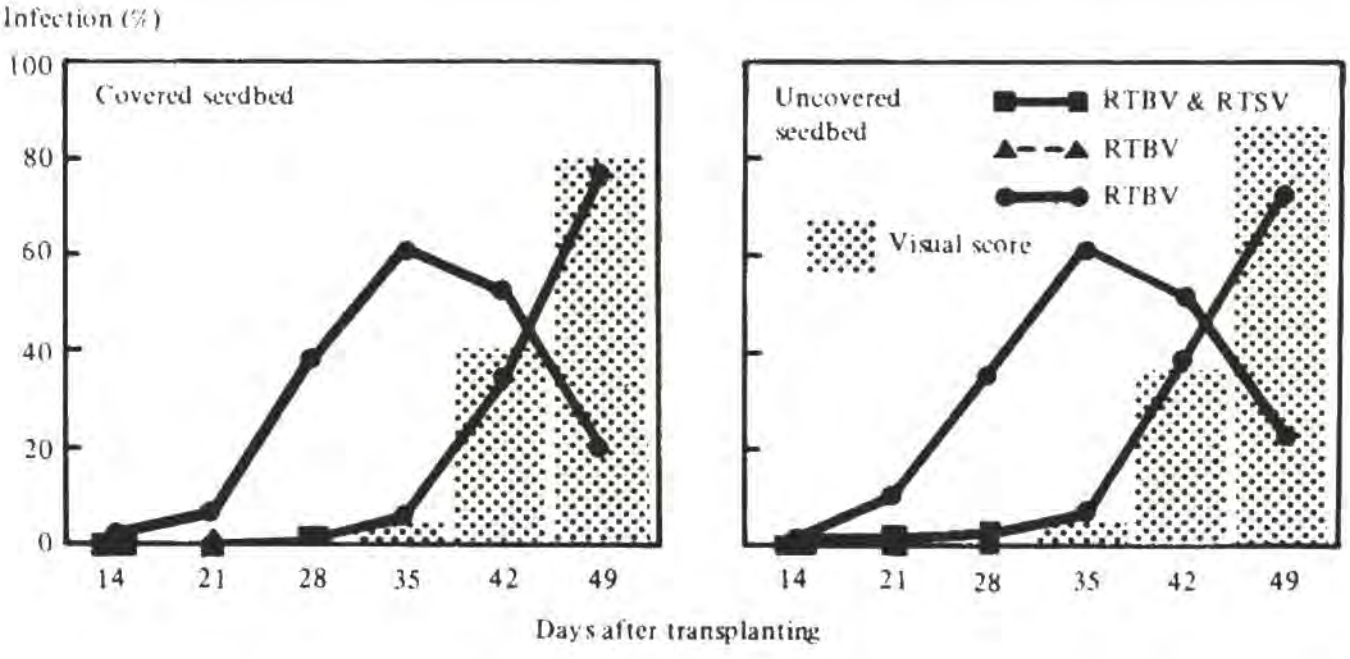

Fig. 1. Percentage infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) as detected by the latex test and visual scores of tungro-infected Taichung Native 1 rice plants from covered and uncovered seedbeds transplanted in the field and scored at different time after transplanting.

Tungro infection on IR62 and IR64

No tungro infection was observed on IR62 and IR64 at 14 DAT. Infection rates of 2.19 and 2.83% were recorded at 28 DAT and increased to 3.50 and 6.85% at 47 DAT. In IR64, difference in infection levels occurred in all observation dates (Table 3).

Table 2. Average number of rice green leafhopper per hill in plots planted to Taichung Native 1 from covered and uncovered seedbeds at different time after transplanting.

Treatment	*Days after transplanting*			
	28	*35*	*42*	*49*
Covered seedbed	1.45 a[1]	0.86 a	0.89 a	0.98 a
Uncovered seedbed	1.37 a	1.02 a	1.04 a	1.09 a

[1]In a column, means having a common letter are not significantly different by DMRT at the 5% level.

Table 3. Average tungro infection in IR62 and IR64 rice varieties at different days after transplanting.

Variety	*Days after transplanting*		
	14	*28*	*47*
IR62	0 a[1] (a)[2]	2.19 a(ab)	3.50 a(b)
IR64	0 a (a)	2.83 a(b)	6.85 b(c)

[1]In a column, means having a common letter are not significantly different by DMRT at the 5% level.

[2]In a row, means having a common letter are not significantly different by DMRT at the 5% level.

Spread of tungro infection

The spatial spread of tungro infection on TN1, IR36, and IR54 plants was studied in the field. No difference in the occurrence of infection in the three distance classes was obtained in all varieties at 37 DAT, although higher levels of infection was recorded on TNI plants (Table 4). At 44 DAT, more direct neighbor hills of TN1 plants were infected than those of diagonal or distant hills while infection rates of the three distance classes in IR36 and IR54 plants did not differ. However, infection rates between direct neighbor and distant hills differed among the varieties. More diagonal hills of TN1 plants were infected than IR36 and IR54 plants. At 44 DAT, the same results were obtained.

In a week's time, viruliferous *N. virescene* under controlled conditions in a field cage infect rice plants close to the virus source (Fig. 2). The 20 leafhoppers were able to infect an average of 11 rice plants with both RTBV and RTSV and 1.25 plants with either RTBV or RTSV. The farthest distance an infected plant was

Table 4. Percentage tungro infection in three distance classes from infected hill of three varieties with different levels of resistance to the rice green leafhopper at different days after transplanting

DAT[1]	*Distance classes*	*TNI*	*Variety*[2] *IR36*	*IR54*
37	Direct neighbor	1.78 a[3](a)[4]	0.52 a(b)	0 a(b)
	Diagonal	1.31 a (a)	0.20 a(b)	0 a(b)
	Distant	1.46 a (a)	0.46 a(b)	0 a(b)
44	Direct neighbor	2.95 a (a)	0.82 a(b)	0 a(c)
	Diagonal	1.56 b (a)	0.39 a(b)	0 a(b)
	Distant	1.91 b (a)	0.81 a(b)	0 a(c)
51	Direct neighbor	4.04 a (a)	1.48 a(b)	0 a(c)
	Diagonal	1.78 b (a)	0.72 a(b)	0 a(b)
	Distant	2.06 b (a)	1.25 a(b)	0 a(c)

[1]Days after transplanting.

[2]Reaction to green leafhopper of TNI-susceptible, IR36 – moderately resistant, and IR54-resistant (Heinrichs et al., 1985).

[3]In a column, means having a common letter are not significantly different by DMRT at the 5% level.

[4]In a row, means having a common letter are not significantly different by DMRT at the 5% level.

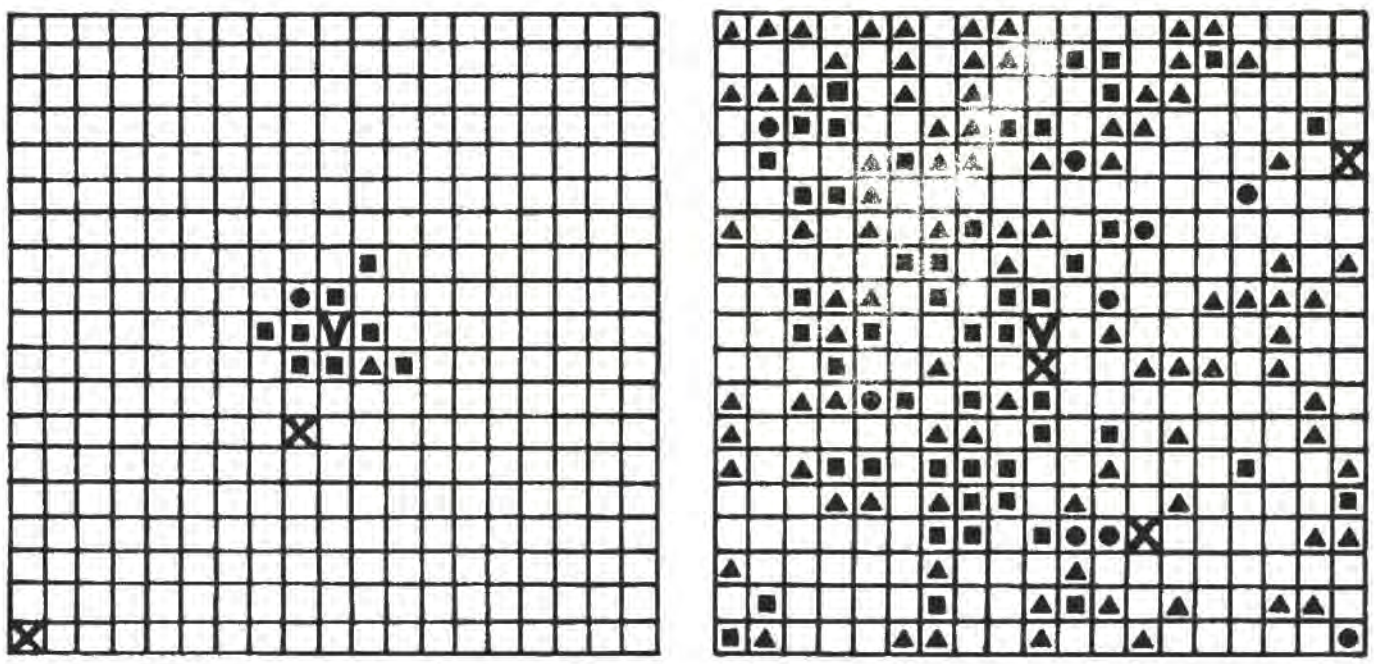

Fig. 2. Representative plots showing the spread of tungro infection by 20 RTBV and RTSV-viruliferous *N. virescens* in 7 days under controlled conditions in field cage (left) and under natural conditions at 30 DAT (right). Symbols: ■ – plants showing symptoms infected with both RTBV, and RTSV, and ● = with RTBV; ▲ = plants without symptoms but infected with RTSV; X = dead plant; and V = virus source.

recorded from the virus source was 128 cm. However under natural conditions, no distinct pattern of spread occurred.

Discussion

Since RTBV and RTSV were incriminated with the tungro disease, little information on the role of these viruses on tungro epidemiology is available.

Results of experiments in the covered and uncovered seedbeds showed that tungro infection, although very low, occurred in plots planted for the uncovered seedlings. Similar results were obtained in another trial wherein infection with RTSV at low rate was observed in plots planted for uncovered seedlings. These are indirect evidences that demonstrate tungro infection occurred, to some extent, in the seedbed. However, after transplanting in the field, no difference in infection rates with any of the viruses were observed between plots planted for the covered and uncovered seedlings. RTSV infection reached its peak within a month after transplanting and gradually decreased thereafter. Meanwhile, plants infected with both RTBV and RTSV increased remarkably within two weeks. The same trend in the development of RTBV and RTSV was obtained in a trial in wet season 1985 (Tiongco, *et al.*, 1986). However, the question on how RTBV gets into the disease cycle remains to be answered.

Although no symptoms were discernible, RTSV infection was detected in the plants by the latex test at 14 DAT. The characteristic symptoms of tungro, like yellowing and stunting, were manifested by the infected plants between 22 and 35 DAT coinciding with the detection of infection with both RTBV and RTSV. Tungro infection in IR62 and IR64 also occurred in the same period of time. These observations concurred with the findings of Hibino *et al.* (1978) that plants infected with both RTBV and RTSV, or RTBV alone showed varying degrees of yellowing and stunting whereas those infected with RTSV were generally symptomless.

RTSV is an important entity in tungro epidemiology. It acts as a "helper" for the transmission of RTBV by leafhopper vectors (Hibino *et al.*, 1978), it occurs widely in farmer's fields (IRRI, 1985; Bajet *et al.*, 1986), it can be transmitted at high rates by leafhoppers that fed on plants infected with RTSV alone (Hibino *et al.*, 1979; Hibino, 1983) and it infects, at high rates, most IR and other rice varieties with resistance to GLH (Daquioag *et al.*, 1984; 1985; Hibino *et al.*, 1987). This study showed RTSV occurred in the seedbed and in newly transplanted fields as a latent virus which limits diagnosis.

Results showed viruliferous leafhoppers in field cages spread tungro to rice plants close to the virus source. The distance of 128 cm obtained in this study showed the capability of viruliferous *N. virescens* to infect plants from the virus source under controlled conditions in field cages in a week's time.

Reports on movements of rice leafhoppers under different conditions have been made (Ling and Carbonell, 1975; Miyashita *et al.*, 1984). Under greenhouse conditions, Ling (1975) observed that seedlings in the proximity of the virus

source had higher infection rates. Similarly, seedling to seedling movements of *N. virescens* in cages were higher between adjacent seedlings (Ling and Carbonell, 1975). Hence, proximity to the virus source is a factor in the spread of tungro infection. Under natural conditions in the field, other factors can contribute to the increase in the amount and extent of spread which may result to an unexpected outbreak of the disease often with little warnings.

The results of these experiments pointed to RTSV as an important element in the initial stages of tungro infection. The absence of discernible symptoms in plants infected with RTSV limits and delays the diagnosis of infection to at least three weeks after transplanting. Early detection of infection is important for a successful control and the use of serodiagnosis is well suited for this purpose.

Some plants from the uncovered seedbeds in this study became infected. This placed the unprotected seedbeds suspect as the staging point in the spread of the disease. Ling *et al.* (1982) pointed that leafhoppers may move to the seedbeds where they lay eggs and transmit tungro. However, it remains to be seen up to what extent the initial infection in the seedbed influenced the disease spread in the field.

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