Transmission of Rice Gall Dwarf Virus by the Green Rice Leafhopper

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ABSTRACT

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The rice gall dwarf virus was transmitted in a persistent manner by the green rice leafhopper and was also transmitted through leafhopper eggs to 87% of the progeny. Transmission efficiency was the highest in *Nephotettix nigropictus* followed by *N. cincticeps* and *N. malayanus*, while *N. virescens* was an inefficient vector. Incubation period of the virus in *N. nigropictus* ranged from 12 to 18 days at 25 C. Transmission efficiency of *N. nigropictus* increased as the acquisition access period was increased, reaching a maximum of 96% with a 12-day acquisition access period.

Rice gall dwarf virus (RGDV) is a leafhopper-borne virus recently described by Omura et al (8). Characteristic symptoms in rice include dark green discoloration of the leaf blades, severe stunting of the plants, and galls along the leaf blades and leaf sheaths. Polyhedral particles about 65 nm in diameter are associated with the disease. In a preliminary test with the green rice leafhoppers *Nephotettix nigropictus* (Stål) and *N. virescens* (Distant), collected in a rice field affected by the disease at the late tillering stage in September 1979 in Uthaitani, central Thailand, the virus was transmitted to healthy rice plants of cultivar RD 1. This report describes the transmission characteristics of the virus by four *Nephotettix* spp.

MATERIALS AND METHODS

RGDV-infected rice plants that had originally been collected in Thailand in September 1979 and brought to the Institute for Plant Virus Research, Tsukuba Science City, Japan, were transferred to the Kyushu National Agricultural Experiment Station in Fukuoka. The virus was transmitted to Taichung Native 1 rice plants using *N. nigropictus*, and the infected plants served as virus source plants for the experiments. We verified the presence of RGDV particles in the plants by electron microscopy.

The leafhoppers originated from stock colonies reared successively on young rice seedlings in an insect chamber at 25 C under continuous fluorescent lighting. Both *N. nigropictus* and *N. virescens* were originally collected in Kagoshima, southern Japan, in 1974; *N. cincticeps* (Uhler) in Fukuoka, southwestern Japan, in 1977; and *N. malayanus* Ishihara & Kawase on Ishigaki Island, Okinawa, Japan, in 1976.

For transmission efficiency tests, groups of each leafhopper species at the third instar stage were given an acquisition access period of 2 days on diseased source plants in plastic cylinders (10 × 16 cm) and maintained at 25 C. They were then allowed to feed individually on rice seedlings of cultivar Reiho, Japonica, in test tubes (1.6 × 18 cm) at 25 C; insects were transferred serially to healthy test seedlings at 2-day intervals for their life span. Total numbers of leafhoppers tested in two experiments were 49 *N. nigropictus*, 134 *N. cincticeps*, 86 *N. malayanus*, and 272 *N. virescens*.

The effect of length of acquisition access on transmission efficiency was determined with *N. nigropictus*, the most efficient vector. Third instars were given acquisition access periods ranging from 3 hr to 12 days on diseased source plants and transferred serially to Reiho seedlings maintained at 25 C under continuous illumination until the death of the insects.

In the test of transovarian passage of the virus, nine females at the third instar nymphal stage were fed on diseased rice plants for 2 days and subsequently paired with healthy males about 1 wk after their emergence. Leafhoppers were confined on Reiho seedlings that were renewed daily at 0800 and 2000 hours until the test female died. Seedlings exposed to the insects from 0800 to 2000 hours were used for collection of progeny, and those exposed from 2000 to 0800 hours were transplanted in a greenhouse to monitor symptom appearance. Eggs were removed from leaf sheaths and placed on moistened filter papers in plastic containers with healthy seedlings in a growth chamber at 25 C. Nymphs collected within 24 hr after hatching were allowed to feed on test rice seedlings in test tubes at 25 C and were transferred individually to new seedlings every 2 days for three consecutive times.

The brown planthopper *Nilaparvata lugens* Stål and the smaller brown planthopper *Laodelphax striatellus* (Fallén) were also tested for ability to transmit RGDV because the former is the vector of rice ragged stunt and grassy stunt viruses and the latter the vector of rice black streaked-dwarf and stripe viruses. Both species had been originally collected in Fukuoka in 1979; test insects were sampled from the colonies of successive generations. Forty individuals of each species at the second instar nymph stage were fed on the RGDV-infected source plants for 3 days, maintained on rice seedlings for 2 wk, and fed individually on Reiho seedlings. Test seedlings were renewed every 2 days until the test insects died. The experiments were conducted in a greenhouse where the temperature ranged between 22 and 30 C.

Throughout the experiments, inoculated seedlings were transplanted in a greenhouse and monitored for symptom development for more than 30 days after inoculation.

RESULTS

Transmission efficiency of *Nephotettix* spp. The transmission pattern suggested a persistent virus-vector relationship (Table 1). Moreover, the transmission pattern was more intermittent than continuous, because about 60% of the infective insects failed to transmit the virus for 2–10 days (transfer at 2-day intervals). The maximum rate of inoculation by the insects (taken as the rate of transmission by individuals among the infective insects) was 94% in *N. nigropictus* at 21–22 days after the acquisition access started. The percentage of inoculation then decreased gradually with time, suggesting intermittence of transmission with advancing age.

N. nigropictus showed the highest rate of transmission, followed by *N. cincticeps*. *N. malayanus* exhibited a slightly

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Table 1. Serial transmission of rice gall dwarf virus by *Nephotettix* spp.

Species Sex	Insects (no.)	Inoculation test feeding on days after virus acquisition started[a]																	
		11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28	29-30	31-32	33-34	35-36	37-38	39-40	41-42	43-44	45-46	47-48
N. nigropictus																				
M	1	−	+	+	−	+	+	+	+	+	−	+	+	+	−					
	2	−	−	−	+	−	+	+	+	+	−	−	−	−						
	3	−	−	−	+	+	+	+	−	+	+	−								
	4	+	+	+	+	+	+	+	+	+	+	+	−	−						
F	1	−	−	−	−	+	+	+	+	−	+	+	+	+	−	+	−			
	2	−	−	−	−	+	+	+	+	−	+	+	−	+	−	−	+	+	+	−
	3	−	−	−	−	+	−	+	+	+	+	X	−	+	+	+	−			
	4	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−				
N. cincticeps																				
M	1	−	−	−	+	−	+	+	+	+	−									
	2	−	−	+	+	+	−	−	−	+	−	−	−							
	3	−	−	+	−	+	+	+	+	+	+	−	+	−	−	−				
	4	−	−	−	+	−	+	+	+	−	−	+	+	−	−					
F	1	−	−	+	+	+	−	−	−	+	−	−								
	2	−	−	+	−	−	+	+	−	+	+	+	−	−	−	+	+	−		
	3	−	+	+	+	−	+	+	+	+	+	+	+	−	−	−				
	4	−	−	−	−	+	+	−	+	+	+	+	+	+	+	−	+	−	−	−
N. malayanus																				
M	1	−	−	−	+	+	−	−	−	+	+	−								
	2	−	−	−	−	−	+	−	+	−	−									
F	1	−	−	+	+	+	+	−	+	−	−	−	−	−						
	2	−	−	−	+	−	+	+	+	+	+	X	−							

[a] + = transmission, − = no transmission, and X = test seedling died.

Table 2. Transmission efficiency and incubation period of rice gall dwarf virus in *Nephotettix* spp.

Species	Insects tested (no.)[a]	Transmission (%)[a]	Incubation period (days)
N. nigropictus	49	51.0	14.5 ± 0.8[b]
N. cincticeps	134	15.7	14.8 ± 1.7
N. malayanus	86	9.4	14.5 ± 2.5
N. virescens	272	0.7[c]	18.0 ± 4.3

[a] Values are averages from two experiments except for *N. malayanus*, which are from one experiment.
[b] At an average confidence limit of ± 95%.
[c] Only two infective individuals were obtained.

Table 3. Transmission of rice gall dwarf virus by *Nephotettix nigropictus* after various acquisition access periods

Acquisition access period	Transmission[a] (%)	Incubation period (days)
3 hr	12	14.0 ± 5.6[b]
6	24	14.6 ± 2.9
12	60	13.6 ± 0.8
1 day	76	13.8 ± 1.2
3	88	14.0 ± 0.8
6	94	14.4 ± 0.9
12	96	14.1 ± 0.7

[a] Each value is for 27–34 insects in one experiment.
[b] At an average confidence limit of ± 95%.

lower rate than that of *N. cincticeps*, and *N. virescens* was a very inefficient vector (Table 2). The time between the starting date of the virus acquisition feeding and the first effective inoculation feeding was taken as the incubation period of the virus in the insects. The incubation period ranged from a minimum of 10 days in *N. nigropictus* to 24 days in *N. cincticeps*. For the majority of the test insects (79%), however, the incubation period ranged from 12 to 18 days. There was no significant difference in the length of the incubation period among three infective species, in spite of differences in their transmission efficiencies. The average incubation period was about 14 days in the three species, whereas the average period of *N. virescens* was 18 days.

Generally, viruliferous insects remained infective throughout their lives.

Acquisition access by *N. nigropictus*. Nymphs of *N. nigropictus* acquired RGDV in 3 hr, the shortest time tested, but the transmission rate was only 12% (Table 3). Transmission efficiency increased as the acquisition access period was increased, reaching 96% with an acquisition access period of 12 days, the longest period tested. Incubation periods following different acquisition access periods did not differ significantly; the incubation period ranged from 11 to 23 days and averaged about 14 days. Males and females of *N. nigropictus* were compared for their ability to transmit RGDV in given acquisition feedings at the nymphal stage. The combined results indicated that a larger number of females than males became infective, particularly when the acquisition access period was short (34% of males vs. 52% of females for 3-hr to 1-day acquisition access; 91% of males vs. 92% of females for 3- to 12-day acquisition access).

Transovarian transmission in *N. nigropictus*. Progeny from females that had acquired RGDV were considered congenitally infected if they could transmit the virus without feeding on diseased rice. Viruliferous females produced both infective and noninfective progeny (Fig. 1), whereas nonviruliferous females produced only noninfective progeny. Infectivities of each brood produced by viruliferous females ranged from 66.7 to 92.8% and averaged 86.9%. In spite of the intermittent pattern of virus transmission by the viruliferous females, they produced infective progeny every day, and the progeny showed an intermittent transmission pattern as first instar nymphs.

Transmission by planthoppers. No transmission was obtained with males or females of *Nilaparvata lugens* or *L. striatellus*, even though all test insects survived at least 20 days after onset of the virus acquisition period.

DISCUSSION

Rice gall dwarf can be distinguished from the 15 known virus and viruslike diseases of rice on the basis of symptomatology, morphology, and serologic relationships of the causal agents (8). The present study provides further basis for distinguishing rice gall dwarf. RGDV belongs to the plant reovirus group that also includes rice ragged stunt, black-streaked dwarf, and dwarf viruses; however, *Nilaparvata lugens*, the vector of rice ragged stunt virus, and *L. striatellus*, the vector of rice black-streaked dwarf virus, did not transmit RGDV. Further, while RGDV was transmitted by *N. nigropictus, N. cincticeps, N. malayanus* and *N. virescens*, only the first two are vectors of rice dwarf virus (1,7). *N. virescens* (9) and *N. malayanus* (Inoue, *unpublished*) are unable to transmit rice dwarf virus, whereas *Recilia dorsalis* (Motschulsky) is the vector of both RGDV (6) and rice dwarf virus (2).

Previously, transovarian transmission

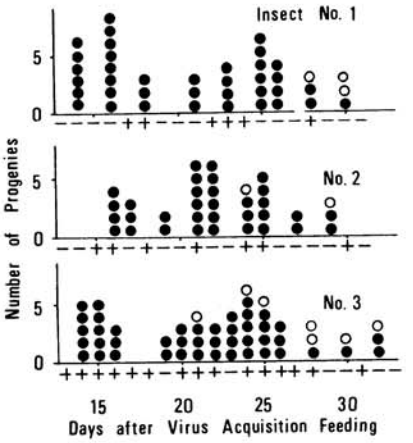

Fig. 1. Infective (●) and healthy (o) progeny from three *Nephotettix nigropictus* females given 2-day acquisition access to plants infected with rice gall dwarf virus. Transmission by the females in serial tests is indicated by + (transmission) and − (no transmission).

has been shown for three rice viruses: rice dwarf virus by *R. dorsalis* (1), *N. cincticeps* (1), and *N. nigropictus* (7); rice stripe virus by *L. striatellus* (5), *Unkanodes (Ribautodelphax) albifascia* (Matsumura) (4,10), and *U. sapporonus* (Matsumura) (10); and hoja blanca virus by *Sogatodes oryzicola* (Muir) (3). RGDV is thus the fourth rice virus for which transovarian transmission has been documented. Such transmission is probably of great importance in epidemiology of rice gall dwarf and perpetuation of the virus.

In general, epidemics of rice virus diseases in Asia—such as tungro in the tropics, transitory yellowing in subtropical and northern tropical regions, and dwarf in the temperate regions—are closely associated with the distribution and prevalence of highly efficient green rice leafhopper vectors. Thus far, rice gall dwarf has occurred sporadically in central Thailand, but the disease might become important because of the predominance of *N. nigropictus* in subtropical and northern tropical regions.

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