Infective Capacity of *Nephotettix* spp. (Hemiptera : Cicadellidae) in the Transmission of Rice Waika Virus¹

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After a 1-day acquisition access period sequential transfers of infective *Nephotettix virescens* to rice seedlings at 30-min intervals for 15 hr indicated an intermittent or erratic pattern of transmission of rice waika virus. The transmission frequency of individual insects averaged 6.3, ranging from 1 to 16 out of 30 transfers. When infective *N. virescens* were transferred at 1-hr intervals for 15 hr, most of the insects displayed a similar intermittent or erratic transmission, although a few insects were consistent transmitters. Transmission frequency averaged 7.4, ranging from 2 to 15. Frequency of transmission was less for *N. cincticeps*, although the pattern of transmission was nearly the same. There was no significant difference in the frequency of transmission between 30-min and 1-hr transfer intervals for *N. virescens*. When different numbers of seedlings were given to a single insect in a tube, an increase in the number of seedlings exposed to the insects did not always result in a corresponding increase in the number of seedlings that became infected.

INTRODUCTION

Leafhopper-borne rice waika virus (RWV) is semipersistent in transmission (HIRAO and INOUE, 1979). Recently, LING and TRONGCO (1977) have proposed the term "transitory" to describe the semipersistent relationship between rice tungro virus and its leafhopper vector. Their definition "transitory" is equally applicable to the vector-virus relationship of RWV. Four species of leafhopper belonging to the genus *Nephotettix* are vectors of RWV as reviewed by FURUTA (1977), and differences in transmission efficiency among these species have been reported by HIRAO and INOUE (1978).

Transmission of RWV by the leafhoppers is characterized by no definite latent period and a short retention period measured in hours or days (HIRAO and INOUE, 1979). This paper reports on the infective capacity of *Nephotettix* leafhoppers to transmit RWV when the insects are transferred sequentially to the test plants at short intervals during a given period. The term "infective capacity"—a quantitative aspect of insect transmission, has been proposed by LING (1976).

MATERIALS AND METHODS

Two species of leafhopper vector, *N. virescens* DISTANT and *N. cincticeps* UHLER,

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were used in this study. These leafhoppers were from stocks reared on young rice seedlings in an insect chamber at $25\pm 1^{\circ}\text{C}$ with continuous fluorescent lighting. *N. virescens* was originally collected from Kagoshima, southern Kyushu, in 1974 and *N. cincticeps* from our experimental fields in the late-autumn of 1978. Young adult females of both species within 10 days after emergence were used. Virus acquisition was carried out by confining the insects in groups in a cylindrical screened cage (30 mm $\times$ 200 mm) on the entire foliar part of the infected source plants. Immediately after the acquisition feeding period, the insects were transferred to healthy rice seedlings.

In the experiments on sequential transfers, individual insects following a 1-day acquisition access period were transferred at 30-min or 1-hr intervals over a 15-hr period to new test tubes (16 mm $\times$ 180 mm), each containing a healthy rice seedling. To investigate infective capacity during a given time, different numbers of rice seedlings were exposed to individual insects for 12 hr following a 1-day acquisition access period. By disturbing the insect with a stick, each insect was forced to move to other seedlings in the tube once an hour. A test tube (16 mm $\times$ 180 mm) was used for a single seedling per insect per tube, and a larger tube (30 mm $\times$ 200 mm) was used for more than three seedlings.

Throughout the experiments, acquisition and inoculation feedings were conducted at a constant temperature of 25°C with continuous fluorescent lighting. RWV-infected rice plants of cv. Reiho, susceptible to RWV, were used as a source of the virus for the acquisition feeding, and test plants of that cultivar at the 1- and 1.5-leaf stage were used for the inoculation feeding.

Inoculated plants were transplanted into pots (160 mm $\times$ 190 mm); three inoculated plants and one non-inoculated control plant per pot. They were then kept in a greenhouse, and transmission records were taken 50 days after inoculation when typical virus symptoms developed on the plants.

RESULTS

Pattern and frequency of sequential transmission by individual insects

Table 1 shows the results of 30 sequential transfers of individual *N. virescens* during a 15-hr period: 63% (19/30) of the insects became infective after a 1-day acquisition access period and transmitted RWV to 21% (119/570) of the plants exposed to them.

The transmission records showed considerable variation in the transmission pattern of individual insects. Most of the insects transmitted in an intermittent or erratic manner. The transmission frequency of individual insects averaged 6.3 ± 4.5 (SD), ranging from 1 to 16 out of 30 transfers in a 15-hr period. The maximum number of consecutive transmissions in the series was six by insect no. 2, followed by five by insect no. 5. Two insects (nos. 2 and 14) transmitted the virus during the first 30-min following acquisition feeding, and 53% of insects transmitted the virus for the first time within 2 hr of the end of the acquisition feeding and 84% (16/19) within 5 hr.

In order to determine transmission frequency following acquisition feeding, the 15-hr period was divided into three 5-hr intervals. The results showed that there was no difference in transmission frequency between the first (47 transmissions) and the second periods (55 transmissions), but that transmission frequency decreased in the last 5-hr period (17 transmissions).

Table 2 shows the results of 15 transfers of *N. cincticeps* and *N. virescens* at 1-hr

Table 1. PATTERN AND FREQUENCY OF RWV TRANSMISSION BY INDIVIDUAL *N. virescens*
(♀) TRANSFERRED SEQUENTIALLY AT 30-MIN INTERVALS FOR 15 HR AT 25°C
FOLLOWING A 1-DAY ACQUISITION ACCESS PERIOD

Insect no. ^a	Transmission record at 30-min transfer intervals ^b															
	0.5	1	2	3	4	5	6	7	8							
1	—	+	+	—	—	—	—	+	+	+	+	—	+	+	—	+
2	+	+	—	—	—	—	+	+	+	—	+	+	+	+	+	—
3	—	—	—	+	+	+	+	—	+	—	—	+	—	+	+	—
4	—	+	—	+	—	—	+	+	—	+	+	—	+	—	+	—
5	—	—	+	—	—	—	—	+	+	+	+	+	—	+	+	—
6	—	—	+	—	+	+	—	—	—	—	+	—	—	—	—	+
7	—	—	+	—	—	—	—	—	—	—	+	—	+	—	+	—
8	—	—	—	—	—	+	—	+	+	+	—	—	+	—	+	—
9	—	+	—	+	—	—	+	—	—	—	—	—	+	—	+	—
10	—	—	—	—	+	+	—	—	+	+	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	+	—	—	—	+	+	—
12	—	—	—	—	+	—	—	—	—	+	+	+	—	+	—	—
13	—	—	+	—	—	—	—	—	—	—	—	—	+	—	+	—
14	+	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
16	—	—	—	—	—	—	—	—	—	—	+	—	+	—	—	—
17	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	+
18	—	—	—	—	—	+	—	—	—	—	—	+	—	—	—	—
19	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—
Total	2	4	5	3	4	6	4	6	6	7	8	5	9	6	9	2

Insect no.	Transmission record at 30-min transfer intervals													Frequency of transmission
	9	10	11	12	13	14	15							
1	+	+	—	—	+	+	+	—	—	+	—	+	—	16
2	—	+	—	—	—	—	+	—	+	+	—	—	—	15
3	+	—	—	+	—	+	+	—	—	—	—	—	—	13
4	—	—	—	—	+	—	—	—	—	—	—	+	—	10
5	—	+	—	—	—	—	—	—	—	—	—	—	—	9
6	+	+	—	—	—	—	—	—	—	—	—	—	—	7
7	+	—	+	—	—	—	—	—	—	—	—	—	—	6
8	—	—	—	—	—	—	—	—	—	—	—	—	—	6
9	—	—	—	—	—	—	—	—	—	—	—	—	—	6
10	—	—	—	—	—	—	—	—	—	—	+	—	—	5
11	—	—	—	—	—	+	—	—	—	—	—	—	—	5
12	—	—	—	—	—	—	—	—	—	—	—	—	—	5
13	—	—	—	—	+	—	—	—	—	—	—	—	—	4
14	—	—	—	+	—	—	—	—	—	—	—	—	—	3
15	—	—	+	—	—	—	—	—	—	—	—	—	—	2
16	—	—	—	—	—	—	—	—	—	—	—	—	—	2
17	—	—	—	—	—	—	—	—	—	—	—	—	—	2
18	—	—	—	—	—	—	—	—	—	—	—	—	—	2
19	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Total	4	4	2	2	3	3	3	0	1	2	1	2	0	119

^a Only infective insects are presented here and arranged in descending order of transmission frequency.

^b Symbols: + = transmission; — = no transmission.

TABLE 2. PATTERN AND FREQUENCY OF RWV TRANSMISSION BY INDIVIDUAL *Nephotettix* LEAFHOPPERS (♀) TRANSFERRED SEQUENTIALLY AT 1-HR INTERVALS FOR 15-HR AT 25°C FOLLOWING A 1-DAY ACQUISITION ACCESS PERIOD

Insect species and no. ^a	Transmission record at 1-hr transfer intervals ^b															Frequency of transmission
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
<i>N. cincticeps</i> (Experiment 1, 12/36) ^c																
1	+	+	+	—	—	+	+	—	—	+	—	+	—	—	+	8
2	+	—	+	+	—	+	+	—	—	—	—	—	—	+	+	7
3	+	—	—	—	—	+	—	—	—	—	+	+	+	—	+	6
4	+	—	—	—	+	+	—	—	+	—	—	—	—	—	—	4
5	+	+	+	—	—	—	—	—	—	—	—	—	—	+	—	4
6	+	+	—	—	—	—	—	—	—	+	—	—	+	—	—	4
7	—	+	+	+	—	—	+	—	—	—	—	—	—	—	—	4
8	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	3
9	+	—	—	—	+	—	—	—	—	+	—	—	—	—	—	3
10	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	2
11	—	—	—	—	+	—	—	—	—	—	+	—	—	—	—	2
12	+	—	—	—	—	—	—	—	+	—	—	—	—	—	—	2
Total	9	4	4	2	3	4	3	0	3	4	3	2	3	2	3	49
<i>N. virescens</i> (27/30)																
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
2	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	14
3	+	—	+	+	+	+	+	—	+	+	+	+	+	+	+	13
4	—	+	+	—	+	+	+	+	+	+	+	+	+	+	+	13
5	—	+	—	+	+	—	+	+	+	+	+	+	+	—	+	11
6	+	—	+	—	+	+	+	+	—	+	+	+	+	—	+	11
7	+	+	+	+	+	+	—	+	—	+	—	+	—	+	—	10
8	+	+	+	+	+	—	—	+	—	+	—	+	—	+	—	9
9	—	—	—	+	+	—	+	—	+	+	+	+	—	—	+	8
10	+	+	—	+	—	—	+	+	+	+	—	+	—	—	—	8
11	+	—	—	+	+	—	—	+	+	—	+	—	+	—	+	8
12	+	—	—	+	+	+	—	+	—	—	+	—	+	+	—	8
13	—	—	+	+	+	—	+	—	+	+	—	—	—	+	—	7
14	—	—	+	+	—	—	+	—	+	—	—	+	+	—	+	7
15	—	—	—	+	+	—	+	+	+	—	—	+	—	—	—	6
16	—	—	—	+	+	—	—	—	+	—	+	+	—	—	+	6
17	+	+	—	—	+	+	—	—	+	+	—	—	—	—	—	6
18	—	+	—	+	—	—	—	—	+	—	+	+	—	—	—	5
19	+	+	—	—	—	—	—	—	—	—	+	+	+	—	—	5
20	—	—	+	—	+	+	—	—	+	—	—	—	—	—	+	5
21	+	+	—	—	+	—	+	—	—	+	—	—	—	—	—	5
22	—	—	—	+	+	—	+	—	+	—	+	—	—	—	—	5
23	+	—	+	—	+	—	+	—	—	—	—	—	—	—	—	4
24	—	+	—	—	—	—	+	—	—	—	—	—	+	+	—	4
25	+	—	—	—	—	+	—	—	+	—	—	—	—	—	—	3
26	—	+	—	+	+	—	—	—	—	—	—	—	—	—	—	3
27	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	2
Total	14	13	11	17	20	10	14	11	17	13	14	16	11	9	11	201

^a Only infective insects are presented here and arranged in descending order of transmission frequency.

^b Symbols: + = transmission; - = no transmission.

^c No. of infective insects/no. of insects tested.

intervals. Two experiments were carried out using *N. cincticeps* at different times on different source plants, but as the results were similar, only the data from Experiment 1 are presented in Table 2. There was a distinct difference between the two species in the percentage of insects which became infective and the percentage of plants they infected: 90% (27/30) of the *N. virescens* females became infective and transmitted the virus to 50% (201/405) of the plants they fed on, while only 40% (25/63) of the *N. cincticeps* females acquired the virus and infected 27% (49/180) of the plants exposed to them.

The transmission pattern also varied considerably in individual insects of both species. Most of the insects displayed an intermittent or erratic transmission. The average transmission frequency of *N. cincticeps* in the combined experimental results ($n=25$) was 4.0 ± 1.9 (SD), ranging from 1 to 8 out of the 15 transfers. The maximum number of consecutive transmissions was three, occurring five times by five insects (nos. 1, 3, 5, 7, and 8). For *N. virescens*, the transmission frequency of individual insects varied from 2 to 15, with an average of 7.4 ± 3.5 (SD). Of particular interest are the facts that one *N. virescens* female (no. 1) transmitted every 15 transfers, and that 7 out of 27 insects transmitted more than ten times, showing a fairly constant transmission pattern during a 15-hr period. In both species, transmission occurred more frequently during the first 5-hr period following acquisition feeding, decreasing to some extent in the remaining 10 hr.

Number of rice seedlings infected by an insect under confined conditions

Table 3 shows that an increase in the number of seedlings exposed to a single infective insect did not always result in a corresponding increase in the number of seedlings that became infected. For example, the number of infected seedlings in the 12-seedling plot was only twice that in the 3-seedling plot. The maximum number of infected seedlings was 3, 4, 6, and 8 seedlings in the 3-, 6-, 9-, and 12-seedling plots, respectively. The standard deviation values indicate that variation in infective capacity among the individual insects is greater in plots with 9 or more seedlings.

DISCUSSION

Throughout the experiments, the infective capacity of *N. virescens* was greater than that of *N. cincticeps*, reflecting a difference in transmission efficiency between the

Table 3. NUMBER OF RICE SEEDLINGS INFECTED BY AN INSECT CONFINED IN A TEST TUBE WITH VARIOUS NUMBERS OF SEEDLINGS FOR 12 HR AT 25°C

No. seedlings/ insect/tube	<i>N. cincticeps</i>		<i>N. virescens</i>	
	No. insects tested ^a	Avg no. infected seedlings with SD	No. insects tested	Avg no. infected seedlings with SD
1	9	1.0	14	1.0
3	14	1.9 ± 0.3	15	2.2 ± 0.8
6	13	2.0 ± 0.5	15	2.6 ± 0.9
9	15	3.1 ± 1.4	13	3.3 ± 1.4
12	13	3.2 ± 1.3	16	4.7 ± 1.5

^a Only infective insects out of the 17 insects tested in each plot are presented.

two species (HIRAO and INOUE, 1978), although considerable variation in infective capacity was observed in individual insects of both species. The most significant finding is that infectivity is unaffected by the length of time the insect has for transmission (i.e., 30-min or 1-hr). Thus, even though an insect may spend only a very short time on a plant, infective capacity in terms of the total number of plants infected does not increase during a given period. In other words, when the transmission interval shortens from 1-hr to 30-min, the percentage of plants infected decreases (cf. Tables 1 and 2). This can be explained by the length of the transfer time. The transmission threshold time for *N. cincticeps* adults at 23°C is less than 30 min, but efficiency of transmission was better with a 1-hr inoculation access time than with a 30-min time (HIRAO and INOUE, 1979). Thus, a transfer interval of 30 min is probably not sufficient for the completion of RWV transmission. In view of this, the minimum time required for RWV transmission by individual insects should be examined precisely using the electrical measurement system (EMS) (McLEAN, 1977). *N. cincticeps* is said to be a phloem feeder (NAITO and MASAKI, 1967a).

In studies of *N. virescens* as the vector of rice tungro disease, a similar virus to RWV in vector-virus relationship (SARTO, 1977), LING (1976) reported that the maximum infective capacity (theoretical) was 30 infected seedlings per insect per day based on 30-min transfer intervals which gave the best results from trials with five different intervals. According to his methods, the maximum infective capacity in RWV transmission can be calculated to be 24 and 13 infected seedlings per insect per day for *N. virescens* and *N. cincticeps*, respectively, based on the results of 1-hr transfer intervals. The former is twice as large as the latter, but these values, of course, are experimental and theoretical, and assume that the infectivity of the first 15-hr period does not change for the rest of the day. LING and TIONGCO (1977) noted the close dependence between infective capacity and temperature.

The RWV is retained by leafhoppers for up to 2 or 3 days at 25°C, with a half-life of about 12 hr, and the retention period depends on the degree of vector efficiency and temperatures (HIRAO and INOUE, 1978). In the hourly transfer experiments, there was only a slight decrease in the infectivity of both vector species over the 15 hr in which the insects were tested.

With regard to the feeding behavior of *N. cincticeps* on rice plants, NAITO and MASAKI (1967b) reported that the average probing time was 29.2 min, and that the average non-probing time was only 5.1 min. Thus, 85% of the time during a given period is accounted for by feeding. Their data indicate that probing is very frequent from one site to another on the plants. This feeding behavior would be related closely to the infective capacity of the leafhoppers to transmit RWV under field conditions, although the virus retention period is limited to a short duration unlike persistent viruses which are retained throughout the life of the insect.

As far as the infective capacity is concerned, *N. virescens* is a more important transmitter of RWV disease than *N. cincticeps*. However, the latter plays an important role in the incidence of the disease because of differences in the distribution of the insect species (HIRAO and INOUE, 1978).

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