Effects of Temperature on the Transmission of Rice Waika Virus by *Nephotettix cincticeps* UHLER (Hemiptera: Cicadellidae)

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Increasing the temperature and lengthening the acquisition access period improved the ability of *Nephotettix cincticeps* UHLER to acquire rice waika virus. Acquisition efficiency significantly increased up to a 24-hr access period and from 20 to 25°C. The maximum rate of transmission was obtained with a 48-hr acquisition access period at 30°C. Similarly, a greater efficiency of inoculation was obtained at higher temperature. In sequential transfer tests, temperature altered virus retention in the adults from only 1.5 days at 30°C to approximately 7 days at 15°C. When overwintering nymphs (late-instar) were examined, the maximum retention period increased to 3 weeks at 0–7°C. The rate of loss of infectivity by the vector showed nearly the same trend at alternating temperatures of 15 to 30°C and 30 to 15°C at 12-hr transfer intervals as at 30°C constant temperature.

**INTRODUCTION**

Temperature is a feature of the environment commonly studied in biological processes. The effect of temperature on the transmission of many plant viruses by vector insects has also been studied. There are two semipersistent leafhopper-borne viruses of rice in the world, rice tungro virus (RTV) in the tropics of Asia and rice waika virus (RWV) in Japan. Similarities between the two viruses have been studied from many aspects such as the virus-vector relationship, serological examination, electron microscopic observation of virus particles, and symptomatology on differential rice varieties (*SATO, 1977; YAMASHITA et al., 1977; HIRAO and INOUE, 1978, 1979; IWASAKI et al., 1978)*.

Recently, *LING* and *TONGCO* (1977, 1979) have reported the results of extensive studies on the relationship between RTV transmission and temperature, and they have proposed the term "transitory" instead of "semipersistent" to explain the RTV-vector relationship. Their proposal is based mainly on new findings showing the prolongation of the retention period of the virus in the leafhopper vector, *Nephotettix virescens* DISTANT, at low temperatures.

Four species of leafhopper are known to be vectors of RWV. Among them *N. cincticeps* UHLER, an intermediate efficient vector, is the most important in the incidence of the disease (*HIRAO and INOUE, 1978*). In this paper, we examine the effect of temperature on the transmission of RWV (acquisition, inoculation, and retention) by *N. cincticeps*. This study also examines whether overwintering leafhoppers could retain the virus throughout the winter season in relation to the annual cycle of the
virus.

MATERIALS AND METHODS

*N. cincticeps* used were collected from our experimental fields in the late-autumn of 1978, and reared on young rice seedlings in an insect chamber at 25±1°C with continuous fluorescent lighting. Rice plants of cv. Reiho, susceptible to RWV, were used for rearing the insects, for virus source, and as test plants. Test plants were generally in the 1- and 1.5-leaf stage at the time of inoculation. Male adults within 10 days of emergence were used throughout the experiments. Virus acquisition was carried out by confining the insects in groups in a cylindrical screened cage (30 mm × 200 mm) on the entire foliar part of the source plants. Immediately after acquisition feeding, the insects were transferred to the test plants using a test tube (16 mm × 180 mm). All acquisition and inoculation feeding periods were in incubators at constant temperatures with continuous fluorescent lighting. Unless otherwise indicated, fluctuations of temperature in the incubators were ±1°C.

The effect of temperature on virus acquisition was investigated in combination with different lengths of acquisition access periods. Following the test acquisition access period each at four different temperatures, the insects were given a 2-day inoculation access period at 25°C in all treatments. One and two insects per plant, 30 and 60 insects per treatment, were tested in Experiments 1 and 2, respectively.

In the experiments on virus retention at constant temperatures, after a 2-day acquisition access period at 25°C, the male adults were divided into four groups, and individual insects were transferred to the test plants at intervals of 2 days at 15°C, 1 day at 20°C, and 0.5 day at 25 and 30°C respectively. A second experiment was carried out which was identical to the first, except that retention was tested at alternating temperatures of 15 to 30°C and 30 to 15°C at 12-hr transfer intervals. In these experiments, 25–30 insects per treatment were tested with two replicates.

In relation to the overwintering of the virus in the leafhopper, experiments were designed to determine how long the insects could retain infectivity at low temperatures. The experiments were conducted for three years using field-collected nymphs (late-instar). After a 2-day acquisition access period on the source plants, the insects were then reared on the water foxtail grass (*Alopeculus aequalis* Sonolewski var. *amurensis* Ohwi). The grass is not a host of RWV (Furuta, 1977), but is the most important overwintering plant for *N. cincticeps*. Insect infectivity was tested at weekly intervals with a 2-day inoculation access period at 25°C. All insects once tested were discarded. Other experimental procedures are shown together with the results in Table 1.

Throughout the experiments, inoculated plants were transplanted into pots (160 mm × 190 mm), three inoculated plants and one non-inoculated control plant per pot, and grown in a greenhouse. Transmission records were taken 50 days after infection when distinct disease symptoms appeared on the plants. When a single insect per test plant was placed for the inoculation feed, the percentage of infective plants was used directly to measure the infectivity of insects. In the case of more than two insects per plant, estimated percentages of infective insects (P) were calculated by the following equation (Gibbs and Gower, 1960):

\[
P = 1 - (1 - R/N)^{1/n}
\]
where $N$ is the number of test plants, $R$ the number of infected plants, and $n$ the number of insects placed per plant for the inoculation feed.

**RESULTS**

*Acquisition efficiency*

The combined results of Experiments 1 and 2 are shown in Fig. 1. Acquisition efficiency increased both with lengthening the access period and with increasing temperature over the range tested. No acquisition occurred with a 6-hr access period at 15 and 20°C, while the insects could acquire the virus with a 3-hr access period at 30°C. As shown by the slopes of the curves, acquisition efficiency increased sharply up to a 24-hr access period at all temperatures except 15°C, and thereafter it increased gradually with longer access periods. Acquisition efficiency was greatly improved by increasing the temperature from 20 to 25°C. The efficiency of virus acquisition increased only slightly between 25 and 30°C. Maximum transmission was obtained with a 48-hr acquisition access period at 30°C, the longest period and the highest temperature tested.

*Inoculation efficiency and retention of infectivity at various constant temperatures*

The effect of temperature on inoculation efficiency is shown at the first transfer time in Fig. 2. The results showed that inoculation efficiency increased as temperature increased.

Temperature significantly affected the retention of infectivity; the lower the temperature the longer the retention period. The maximum retention period was approximately 7, 4, 2, and 1.5 days at 15, 20, 25, and 30°C, respectively. The rate of decline in infectivity with time also varied with temperature, being greatest at 30°C and least at 15°C. The rate of loss of infectivity was similar between 25 and 30°C.

![Fig. 1. Effects of temperature combined with the length of acquisition access time on the efficiency of RWV acquisition by *N. cincticeps*. Male adults were given 4 or 5 acquisition access time at 4 temperatures and then transferred individually to test plants for 2 days at 25°C.](image-url)
Of interest is the fact that the rate was most sensitive to changes from 20 to 25°C, compared with the other 5°C changes in temperature.

Retention of infectivity at alternating temperatures

In the experiment with alternating temperatures, the rate of transmission was always higher at 30°C than at 15°C over the period tested (Fig. 3). The rate of loss of infectivity with time followed the same trend in the two tests, and also, this trend was nearly the same as that at the constant temperatures of 25 and 30°C mentioned above.
Temperature and RWV by Leafhopper

Table 1. Retention Period of RWV in N. cincticeps (Overwintering Nymphs) under Low Temperature Conditions

<table>
<thead>
<tr>
<th>Weeks after acquisition feeding</th>
<th>1976 (Mar. 5)(^a)</th>
<th>1977 (Jan. 19)(^b)</th>
<th>1979 (Jan. 9)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transmission %</td>
<td>Transmission %</td>
<td>Transmission %</td>
</tr>
<tr>
<td>0</td>
<td>7/47(^b) 15</td>
<td>18/30(^c) 26</td>
<td>4/30(^b) 13</td>
</tr>
<tr>
<td>1</td>
<td>6/39 15</td>
<td>10/29 14</td>
<td>2/37 5</td>
</tr>
<tr>
<td>2</td>
<td>2/35 6</td>
<td>5/30 6</td>
<td>1/61 2</td>
</tr>
<tr>
<td>3</td>
<td>2/36 6</td>
<td>1/30 1</td>
<td>1/52 2</td>
</tr>
<tr>
<td>4</td>
<td>0/36 0</td>
<td>0/30 0</td>
<td>0/53 0</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>0/30 0</td>
<td>—</td>
</tr>
<tr>
<td>Acquisition</td>
<td>2 days, 15(9.5~21)(^c)</td>
<td>18 hr, 25(^c)C</td>
<td>2 days, 25(^c)C</td>
</tr>
<tr>
<td>Inoculation</td>
<td>2 days, 25(^c)C</td>
<td>2 days, 25(^c)C</td>
<td>2 days, 25(^c)C</td>
</tr>
<tr>
<td>Rearing on grass</td>
<td>7±3(^c), 2ML-16D</td>
<td>5±0.5(^c), 8L-16D</td>
<td>0±0.5(^c), 8L-16D</td>
</tr>
</tbody>
</table>

\(^a\) Date of insect collection.
\(^b\) No. of infective insects/no. of insects tested in the case of 1 insect/test plant.
\(^c\) No. of infective plants/no. of plants tested in the case of 3 insects/test plant. See text for the calculation of % infective insects.

Retention of infectivity at low temperatures

In 1977 and 1979 the test insects were in diapause, while they were post-diapause in 1976. The results showed that virus retention in the insects decreased with time (Table 1). The longest retention period was 3 weeks when the insects were kept at low temperatures. This trend was not influenced by the differences in the dates of insect collection or by temperatures at which the insects were kept on the host grasses.

DISCUSSION

The results show that the following features of virus-vector relationship are common to both RWV and RTV: (1) positive correlation of acquisition and inoculation efficiency with increasing temperature, (2) an increase in infectivity with longer acquisition periods, (3) a retention period of hours or days, depending on temperature, and (4) a prolongation of infectivity time at low temperatures. Thus, it seems that RWV belongs to the same group as RTV and the term "transitory," proposed by Li and Tiongco (1977, 1979), is applicable to the virus-vector relationship of RWV.

Temperature is one factor that directly affects the feeding behavior of insects. Generally, the amount of sap ingested as estimated by the amount of excreted honeydew decreases with decreasing temperature (Ôya, pers. comm.). RWV particles are found only in phloem cells and the concentration of the virus in rice plants is very low when compared with other plant viruses (Saitô, 1977; Yamashita et al., 1977). On the other hand, N. cincticeps is a vascular bundle feeder (Naito and Masaki, 1967). When these factors are taken into consideration, it can be assumed that retarded ingestion due to low temperature gives the insects less chance to successfully penetrate the plant with their stylets and to feed in the phloem tissues. This would result in inefficient acquisition and inoculation. As shown in Fig. 1, temperature significantly affects acquisition efficiency when compared with the length of the feeding
period. In relation to virus transmission, probing behavior of leafhoppers under various conditions should be studied precisely using the electrical measurement system (McLean, 1977).

The retention experiment showed a maximum retention period of 3 weeks at low temperatures of 0–7°C. The average monthly temperature in northern Kyushu is 7.4°C in December and 5.0°C in January. The maximum retention period, therefore, is not sufficient to cover the long winter season, and it appears that RWV cannot overwinter in insects although it may do so in plants. No host plants of RWV other than Oryza plants have not been detected (Furuta, 1977). Recently, Iwasaki et al. (1979) have reported that RWV overwinters in the roots of rice stubbles, and that spring ratoons emerging from them serve as a primary inoculum of the virus in early summer.

REFERENCES


