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Ill-Effects of Rice Dwarf Virus upon Its Vector, *Nephotettix cincticeps* UHLER (Hemiptera : Deltocephalidae), and Its Significance for Changes in Relative Abundance of Infected Individuals among Vector Populations

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The deleterious effects of the rice dwarf virus upon its vector *Nephotettix cincticeps*, were studied as a factor affecting inter-generation changes in the percentage of the infected individuals in a vector population.

Developmental period and survival rate of nymphs, longevity of adults, and preoviposition period and fecundity of female adults were compared between infected and non-infected insects reared in isolation under various temperatures. Inter-generation changes in percentage of infected females in experimental populations were also examined at 20°C, 25°C, and 30°C. Virulency of insects was tested by using the rice seedling as a test plant or by the serological method.

The virus was harmful to the vector with respect to all biotic performances examined, especially, to the fecundity of females. An appreciable reduction in fecundity of infected females was observed as compared with that of non-infected ones in summer or in the 3rd generation. Decreases in the percentage of infected females in experimental populations were greater at 25°C and 30°C than at 20°C from the parent to F_1 generation.

Based on these facts, it is suggested that temperature is one of the factors involved in the inter-generation changes in the percentage of infected *N. cincticeps*.

INTRODUCTION

Recently, epidemiological studies of leafhopper-transmitted viruses have advanced in connection with factors that influence dissemination of the viruses (BENNETT, 1967). The extent to which insect-borne virus diseases of the rice plant becomes appreciable in a given locality appears to be determined not only by the abundance of the vectors but also by the percentage of infected insects among the vector population.

The following factors are thought to be responsible for determining the relative abundance of vector individuals infected with viruses of a persistent type: (1) rate of oral acquisition of the virus when the vectors feed on infected plants; (2) rate of transovarial passage, and (3) differential mortality between infected and non-infected insects. The percentage of infected individuals in a vector population tend to increase by oral acquisition of the virus. While it decreases through

transovarial transmission and by harmful effects of the virus on the vector, because no known viruses are transmitted 100 per cent through the eggs to the offspring of the vector, and physiologically beneficial effects of a plant virus upon its vector have scarcely been observed in plant- and leafhoppers.

NASU (1963) suggested that the rice dwarf virus (RDV) is harmful to the vector leafhopper, *Nephotettix cincticeps* UHLER, with respect to survival of nymphs, intensity of diapause, fecundity of female adults, and cytological characteristics of some internal organs of nymphs and adults. This line of evidence was confirmed by SATOMI (cited from MARAMOROSCH, 1968). INOUE and YOSHII (1966) also reported that the adults of *N. cincticeps* infected with RDV had a shorter longevity. In the present paper, we first followed inter-generation changes in the percentage of infected individuals of *N. cincticeps* under natural conditions over four successive generations. Concurrently, we attempted experimentally to assess the degree of ill-effects of the virus upon the vector for caged populations under various thermal conditions. Finally, an interpretation of the inter-generation changes in the relative abundance of infected individuals is proposed based on the effects of the virus upon the vector.

METHODS

Experiment 1 In 1967, 70-100 nymphs of the 5th instar of *N. cincticeps* were collected respectively at early, middle and late periods in each of the four successive generations, from an insecticide-free paddy field in Nangoku City. These nymphs were placed individually in vials (3×30 cm) containing a rice seedling. The vials were kept for 2 days in an insectary room controlled at 25°C, under 24 hr illumination. Each seedling fed by the nymph was then transplanted to an insect-proof greenhouse and examined 30 days after transplantation to detect whether the nymph was infected or not. The nymphs removed from the seedlings were thereafter reared individually at room temperatures on rice seedlings which were renewed every other day. A female adult, on emergence, was paired with a male obtained from the open, and the number of eggs laid by the female together with its life span were recorded every other day when the seedling was renewed.

Experiments 2, 3 and 4 In 1968, a batch of young nymphs from a stock colony of *N. cincticeps,* originally collected from paddy fields in Ino, was reared on rice plants infected with RDV throughout their lives to raise the fraction of the infected insects in the population (No. 1 culture), and another virus-free batch of nymphs, originated from eggs laid by non-infected females (confirmed by the serological method to be explained below), was reared on virus-free rice seedlings (No. 2 culture).

A) Experiment 2.

One hundred individuals of 5th instar from No. 1 culture were reared in isolation under 25°C, 75 per cent R.H., and 24 hr illumination, and their infectivity was checked by the feeding test mentioned above. The number of eggs laid and the longevity of the females were examined daily. The egg masses deposited by each female were placed in a small glass tube (1 × 4.5 cm) with a stripe of filter paper moistened with water to know the duration and the rate of hatching of the eggs. All offsprings from infected females and some randomly selected offsprings

from non-infected females were used for further experiment. These were reared in groups as originated from individual egg-mass until 2nd instar, but reared individually after the 3rd instar. Seedlings were renewed every other day, and the seedlings fed by the nymphs which originated from infected females were planted in the greenhouse to determine the infectivity of the nymphs.

B) Experiment 3.

Ten of the 1st instar nymphs obtained from No. 2 culture were introduced into a vial (3 × 30 cm) with a infected rice seedling which was 30–35 days old after inoculation with RDV. Three groups, each group consisting of 10 vials, were kept for 3 days for oral acquisition of the virus in insectaries controlled at 20°C, 25°C, and 30°C under 16 hr illumination and 75 per cent R.H., respectively. Then all the nymphs were reared individually in glass tubes (3 × 30 cm) with rice seedlings which were renewed every other day under the same thermal conditions throughout their lives. During the course of rearing, of the seedlings which were offered to the insect, as nymph or newly emerged adult, some 4–5 seedlings were planted at arbitrary intervals in the greenhouse to determine whether it became infected or not. Each insect which died at the adult stage was subjected to serological tests.

C) Experiment 4.

The design of the experiment using caged populations was as follows: six groups, each consisting of some 50–60 pairs of adults obtained from No. 1 culture were introduced in Sugimoto's (1969) population cages (48 × 32 × 15 cm) with two-day old rice seedlings. Three groups of paired cages were placed in rooms controlled at 30°C, 25°C, and 20°C respectively, all under 75 per cent R.H., and 16 hr illumination. The females were allowed to oviposit in the cages for 2, 2 and 3 days at 30°C, 25°C and 20°C, respectively and then they were tested individually by serological tests for their infectivity. The cages with the egg-laid rice seedlings were transferred to a room maintained at 30°C, 16 hr illumination, and kept for a further 7 days, which correspond to the duration of the eggs at 30°C, before being returned to the previous rooms in which eggs were deposited. The seedlings which were used for oviposition were kept unchanged for 9–10 days until the hatch of the eggs. Since this duration may exceed the incubation period of RDV in the rice plant, possible oral acquisition of the virus by hatched nymphs could not be excluded. But no visible symptoms, however, were found on the leaves of the seedlings when the hatched nymphs were removed. Three hundred nymphs of the 1st instar were distributed to each cage, to avoid overcrowding. Thereafter, seedlings were renewed twice a week throughout the experiment, to prevent the leafhoppers from acquiring the virus orally through feeding on infected plants. Since the exposure of seedlings for 3–4 days is short enough as compared with incubation period of RDV in the rice plant, it is thought that no insects could acquire RDV orally. On 6th, 7th and 12th days after emergence, which correspond to the preoviposition periods at 30°C, 25°C, and 20°C, respectively, the females were allowed to oviposit eggs of the next generation, then they were tested individually by the serological method.

The serological method used was a hemagglutination test employed by YASUO and YANAGIDA (1963) and KITANI and KISO (1966) for the rice stripe virus and its vector *Laodelphax striatellus*. Antiserum of RDV was furnished by the Phytopathological Laboratory, Shikoku Experimental Station and the 1st Research Division,

Institute for Plant Virus Research. RDV-antibody (γ-globulin)-sensitized sheep red brood cells were sensitive to the juice of infected rice leaves diluted to $1 : 1.6 \times 10^5$ with normal rabbit serum and veronal buffer solution at pH 6.9. A female of *N. cincticeps* was grounded by a glass stick with 0.6 m*l* of normal rabbit serum (veronal buffer solution added) in a centrifuge tube (1.1×10.5 cm), and was centrifuged at 2,500 r.p.m. for 4 min. The supernatant fluid was transferred to another tube and a drop of RDV-antibody sensitized red brood cell suspension was added, and shaken before stocking at 5°C for 24 hr. The star-shaped sedimentation in the positive reaction was easily distinguishable from the doughnut-shaped sedimentation in the negative reaction (Fig. 1).

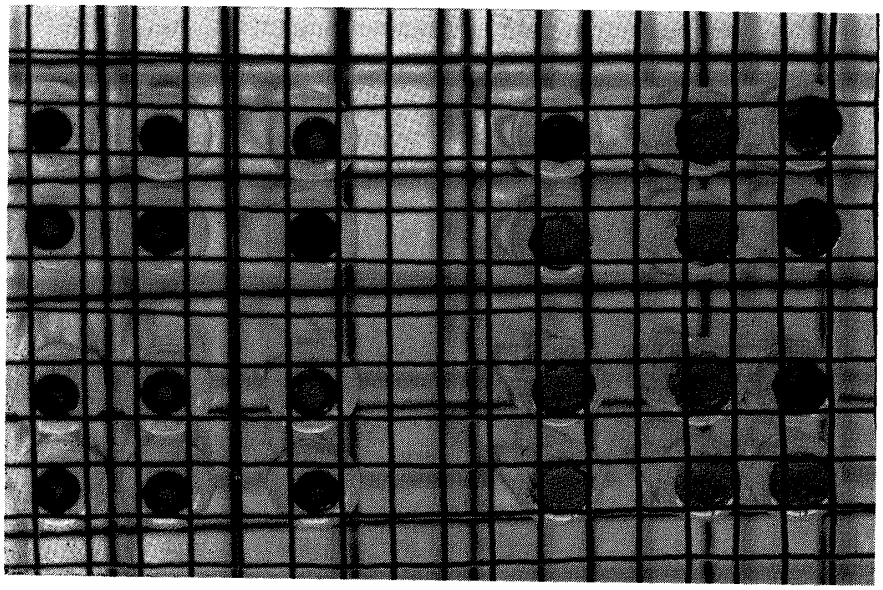

Fig. 1 Sedimentations of red blood cells. Right : positive reaction; Left : negative reaction.

Table 1. COMPARISON OF BIOTIC PERFORMANCES OF FEMALE ADULTS AND SURVIVAL RATES OF EGGS AND NYMPHS BETWEEN INFECTED AND NON-INFECTED *N. cincticeps*

	Infected Mean ± 95% f.l.	Non-infected Mean ± 95% f.l.
Parent female		
No. females reared	7	59
Longevity in days	12.1± 3.7	16.6± 2.1
Preoviposition period in days	7.7± 2.3	6.8± 0.7
Mean fecundity	26.1±41.0	73.7±17.4
Egg		
Hatchability (%)	83[a]	69
Duration of egg stage in days	9.4	8.8
Nymph		
Survival rate (%)	31.1	46.2

Adults were reared individually at 25°C, under 24 hr illumination.

a. 84% of the nymphs from these eggs deposited by the infected females were viruliferous.

Table 2. Oviposition Patterns of Infected and Non-Infected Females at 25°C

	Days after emergence																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30<
Infected																														
1	·	·	·	·	·	12	7	·	15	10	10	12	21	12	7	14	8	·	·	D											
2	·	·	·	·	·	D																								
3	·	·	·	·	·	·	·	D																						
4	·	·	·	D																										
5	·	·	·	·	·	7	·	·	·	D																				
6	·	·	·	·	·	·	·	·	·	D																				
7	·	·	·	·	·	·	·	14	20	·	·	14	D																	
Non-infected																														
1	·	·	·	·	·	11	4	6	7	·	·	3	·	7	·	8	18	·	·	D											
2	·	·	·	·	11	12	10	8	11	4	12	23	·	11	·	·	D														
3	·	·	·	·	4	4	11	6	8	11	9	14	·	·	·	15	·	8	4	17	9	·	2	20	11	7	2	4	·	8
4	·	·	7	5	D																									
5	·	·	·	·	·	6	6	6	10	15	18	10	11	5	·	·	8	·	D											
6	·	·	·	·	·	·	18	15	·	16	19	17	·	22	11	7	·	·	D											
7	·	·	·	·	·	·	6	8	10	·	16	3	15	8	8	21	4	·	5	10	·	4	D							
8	·	·	·	·	·	·	·	·	·	D																				
9	·	·	·	36	10	22	·	24	9	17	·	10	6	D																
10	·	·	·	10	12	·	7	15	15	·	·	11	6	·	·	·	6	23	D											

RESULTS

Deleterious Effects of RDV upon Its Vector, N. cincticeps

Various biotic performances of female adults and survival rates of eggs and nymphs were compared between infected and non-infected insects under a condition of 25°C, 75 per cent R.H., and 24 hr illumination (Expt. 2; Table 1). The longevity of infected females was 4.5 days shorter, but 1 day longer in the preoviposition period, than in the non-infected females. The oviposition period, therefore, became considerably shorter in the infected female. Furthermore, the infected female laid in average 65 per cent fewer eggs than in the non-infected females. The differences in the longevity, preoviposition period, and mean fecundity, however, are not significant ($P=0.05$) between infected and non-infected females (Table 1). In Table 2, oviposition records of the infected females are shown in comparison with those of 10 normal females randomly selected out of 59. Of the 7 infected females, 4 were sterile, and the fecundity of 2 out of the remaining 3 fertile females was abnormally low. Thus, only 1 female showed normal fecundity, notwithstanding that almost all the offsprings from this female were found to be infected transovarially.

Hatchability and duration of eggs were compared between eggs laid by infected and non-infected females (Table 1). The duration of the eggs laid by infected females was slightly longer than that of the eggs from non-infected females but the hatchability was higher in the former than in the latter.

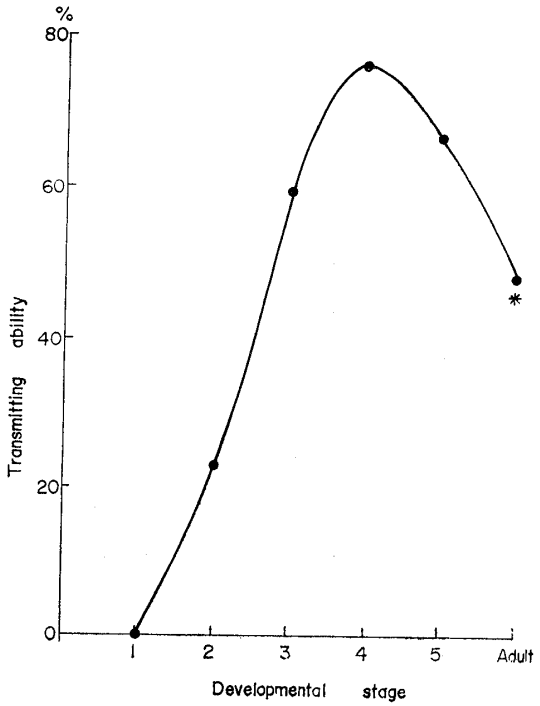

Fig. 2 Relationship between developmental stages and transmitting ability expressed in terms of the percentage of infected seedlings when healthy seedlings were offered to various developmental stages of the infected insect. Data for adults indicated by an asterisk sign were obtained from INOUE and YOSHII (1966).

Table 3. Effects of the Rice Dwarf Virus upon *N. cincticeps* which Acquired the Virus orally at First Instar under Three Thermal Conditions

Temperature	Time of test of virulency		Female					Male		
			No. of insects examined	Developmental period (days)	Longevity (days)	Preoviposition period (days)	Mean fecundity	No. of insects examined	Developmental period (days)	Longevity (days)
20°C	2nd instar within 10 days after emergence	Infected	3	47.7± 7.7[a]	18.0±37.6	12.0±18.0	19.7±55.6	1	45.0	13.0
		Non-infected	27	45.3± 2.6	37.6± 3.9	11.1± 1.3	79.1±19.1	26	43.4± 1.9	36.1± 8.7
	After death by senescence	Infected	6	46.2± 4.1	28.5±16.0	11.8± 1.9	52.7±48.2	7	41.7± 4.7	41.4±18.1
		Non-infected	24	45.2± 1.8	37.4± 4.4	11.1± 1.4	78.3±29.3	20	44.0± 3.9	33.1±10.3
25°C	2nd instar within 6 days after emergence	Infected	9	23.2± 1.8	21.0± 6.3	9.0± 1.7	38.1±30.0 *	4	20.0± 1.6	14.0± 5.7
		Non-infected	28	22.4± 0.9	30.1± 5.8	8.6± 0.8	98.3±28.5 *	24	19.1± 0.8	18.2± 3.6
	After death by senescence	Infected	15	23.1± 1.1	23.9± 5.2 *	8.7± 1.2	53.2±29.0 *	8	19.8± 6.1	15.4± 2.5
		Non-infected	22	22.3± 0.5	34.6± 5.3 *	8.4± 0.8	114.3±30.2 *	15	19.4± 1.0	21.9± 4.8
30°C	2nd instar within 6 days after emergence	Infected	7	15.3± 0.8	16.6± 3.7	7.7± 2.8	25.4±15.6	5	14.8± 2.3	13.6± 5.1
		Non-infected	30	15.6± 0.5	16.5± 2.9	6.2± 0.6	50.3±14.8	29	14.2± 0.4	13.7± 2.5

a. Mean ±95% fiducial limit * $P<0.05$

The survival rate of the nymphs which originated from infected females was lower by 15 per cent than those from non-infected females. In view of the fact that, of the offsprings which originated from infected females and survived until the time of individual rearing, 84 per cent were infected transovarially with the virus, and their low survival rate may be accounted for by the ill-effects of RDV. As a whole, the rate of increase of the infected insects is only 25 per cent of that of non-infected insects, taking into account the difference in fecundity between infected and non-infected females.

Virus transmitting ability of the offspring infected transovarially are shown in Fig. 2. Here, the transmitting ability is expressed by the percentage of the infected rice seedlings to the total number of the test seedlings. Since each test seedling was exposed to the nymphs of 1st and 2nd instars from one egg mass as a group, usually 6-7 individuals, it was impossible to discriminate which individual was viruliferous. In this case, only one individual was tentatively assumed to be infected in calculating the transmitting ability. INOUE and YOSHII's (1966) data for adults which emerged in spring from overwintered nymphs in Nangoku City are also included in figure. The transmitting ability of the infected insects increases rapidly from 1st to 4th instar, but decreases afterwards. This may suggest that rapid multiplication of the virus occurs during young nymphal stages. KIMURA (1962) found that the concentration of the virus in *N. cincticeps* increased most rapidly from 15th to 20th day following injection of the virus. This duration of 15 to 20 days coincides well with the developmental period from 1st to 4th instar at 25°C. Similar changes in transmitting ability related to the developmental stage can be seen in the data of SHINKAI (1962).

A comparative study (Expt. 3) to know the effects of the virus upon its vector when it acquired the virus orally was undertaken under different temperatures (Table 3). Harmful effects of the virus on the biotic performances of its vector were again observed; developmental period, longevity, preoviposition period and fecundity of infected insects were affected irrespective of the rearing temperatures employed, except for the developmental period and longevity of males at 20°C and the longevity of females at 30°C. The differences between infected and non-infected insects in the former three items were slight, but the difference in fecundity was considerably large at every thermal condition. The difference in fecundity between infected and non-infected insects was much greater when females were found to be infected on or soon after emergence than when they were so at the end of life. This indicates that reduction in fecundity caused by the virus is much enhanced when the insects become infected by the time of oviposition.

Effects of RDV upon Female Adults of N. cincticeps in Different Generations of Field Population

Effects of RDV upon female adults of *N. cincticeps* were studied (Expt. 1) in terms of longevity, preoviposition period, and fecundity among four consecutive generations in 1967 (Table 4). The longevity and fecundity for each generation were appreciably lower than those reported by ESAKI and HASHIMOTO (1937), NASU (1963), and KUNO (1968). Deleterious effects of RDV upon females of the 1st, 2nd, and 3rd generations were conspicuous in relation to longevity, preoviposition period, and fecundity. Longevities of infected females were shorter by 4, 2.5,

Table 4. Comparison of Biotic Performances of Female Adults between Infected and Non-Infected Leafhoppers with Regard to Generation

Generation	No. of females reared	% of the infected females among the samples	Longevity in days mean ±95% f.l.	Preoviposition period mean ±95% f.l.	Mean fecundity mean ±95% f.l.		% of infertile females	Ratio of mean fecundity (infected/non-infected)
1st Gen.								
Infected	11	14.5	11.8±5.8	12.0±4.2	20.1±18.9	*	45.5	0.307
Non-infected	65		16.8±2.5	9.5±0.8	65.5± 8.4		33.8	
2nd Gen.								
Infected	9	6.7	4.0±2.4	8.0	0.4± 0.4	*	88.9	0.058
Non-infected	125		6.5±1.9	7.1±0.7	6.5± 1.9		69.6	
3rd Gen.								
Infected	5	4.8	5.2±2.8	——	0.0		100.0	0.000
Non-infected	100		8.6±1.2	9.9±1.8	2.1± 4.1		80.5	
4th Gen.								
Non-infected	45	0.0	20.0±7.9	13.0±3.0	18.5±10.6		68.9	——

Every female adult paired with a male and reared at room temperatures on a rice seedling.

and 3.5 days than those of non-infected, in 1st, 2nd, and 3rd generations, respectively. Furthermore, preoviposition periods of infected females were longer by 2.5 and about 1 day than those of the non-infected, in 1st and 2nd generations, respectively. The mean fecundities of the infected females were significantly ($P=0.05$) lower than those of the non-infected, in both 1st and 2nd generations. The ratio of the mean fecundity of infected females to that of non-infected decreased gradually from 1st to 3rd generation, corresponding to the decrease in the percentage of reproductive females. The fact that the females of summer generations are less fecund than the others was already reported by Esaki and Hashimoto (1937), Nasu (1963), Kuno (1968), and Kiritani et al. (in preparation), and the decreased fecundity was considered to be due partly to intraspecific interference among the insects under higher population densities, and partly to physiological ill-effects of high temperatures in summer (Kiritani et al. *l.c.*). It is possible, therefore, that deleterious effects of the virus on the vectors are enhanced by high temperatures.

Effects of RDV on the Vector under Different Thermal Conditions

An attempt was made to compare the relative rates of increase of infected with that of non-infected insects under three thermal conditions, i. e. 30°C, 25°C, and 20°C (Expt. 4; Table 5 and Fig. 3). Durations for the completion of one generation were 25, 31, and 53 days at 30°C, 25°C, and 20°C, respectively. The mean daily survival rates from hatching to the time when adults were taken out after 2- to 3-day oviposition were highest at 20°C, followed by 25°C, and 30°C in the decreasing order.

Table 5. DURATION REQUIRED FOR COMPLETION OF ONE GENERATION AND SURVIVAL RATES AT DIFFERENT TEMPERATURES

Temperature	Duration required for completion of one generation (days)	Daily survial rate[a]
20°C	53	96.31
25°C	31	95.32
30°C	25	91.94

a. Daily survival rate during the period from hatching to the time when adults were taken out after being allowed to oviposit for 2 days at 25°C and 30°C, and 3 days at 20°C, respectively.

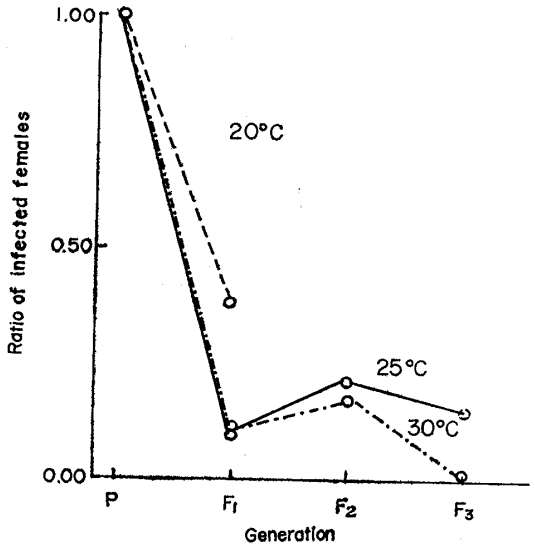

Fig. 3 Changes in the proportion of infected females among the progeny of subsequent generations under three thermal conditions, assuming the initial proportion of infected females in the parental generation as unity.

Changes in the proportion of infected females among the progeny were studied for three consecutive generations at 25°C and 30°C, and for two generations at 20°C (Fig. 3). The greatest decrease in the proportion was observed from the parent to F_1 generation, irrespective of temperature. The rates of decrease at 30°C and 25°C were greater than at 20°C, i. e. 0.89, 0.89, and 0.62 at 30°C, 25°C, and 20°C, respectively. Though the value at 25°C, i. e. 0.89, was higher than the value obtained from Experiment 2, viz., $1-0.25=0.75$, it is considered that the former values were possibly overestimated. The proportion of infected females became nill at 30°C in the 3rd generation, while there were still a few infected females at 25°C.

DISCUSSION

So far, many workers have studied various types of effects of plant disease

agents on their vectors, e. g. the aster-yellows *versus Macrosteles fascifrons* (LITTAU and MARAMOROSCH, 1956), the European wheat striate mosaic *versus Delphacodes pellucida* (WATSON and SHINHA, 1959), the western X-disease *versus Colladonus montanus* (JENSEN 1959; JENSEN et al., 1967), and the dwarf disease of Satsuma orange *versus Geisha distinctissima* (YOSHII and KISO, 1957).

It has been demonstrated that *N. cincticeps* infected with RDV is affected physiologically in various ways, especially most severely in the adult stage. In transovarially infected vector, the virus transmitting ability is highest during old nymphal stages, probably because the virus mostly multiplies in this period. Afterwards, the transmitting ability decreases, while deleterious effects of the virus upon the vector become more severe. On the other hand, probable peak multiplication of the virus comes after emergence of the adult when the vector acquires the virus orally as young nymph (SHINKAI, 1962).

The reduction in fecundity of infected females as compared with that of non-infected ones becomes greater especially in summer, or 3rd generation. One of the causative factors responsible for this reduction in fecundity of the infected female is suspected to be thermal conditions. This was sustained by the observations of experimental populations maintained at different temperatures (See the third section in results).

KŌNO (1966) proposed a formula[1] to describe inter-generation changes in the percentage of viruliferous individuals in a population of *L. striatellus*, the vector of the rice stripe virus. The formula comprises three components, i. e., the probabilities of transovarial transmission (r) and of acquisition by feeding (w) of virus, and of occurrence of infected individuals in the vector population (P). In the case of RDV and *N. cincticeps*, there should be added to KŌNO's formula another component, i. e. relative rate of increase of infected and non-infected insects. The relative rate of increase of infected and non-infected insects is not only a function of thermal condition, but also of the other components involved in KONO's model which would also vary in relation to environmental factors. Therefore, the formula would become more complicated than that for *L. striatellus*.

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1. $P_{n+1}=P_n r(1-w)+w$
where P_n and P_{n+1} are the probability of occurrence of infected individuals in the vector population in nth and $(n+1)$th generations.

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