Technical Bulletin 7
December, 1974

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Tech. Bull.
TARC
No. 7

Tropical Agriculture Research Center
Ministry of Agriculture and Forestry
Nishigahara 2-2-1, Kita-Ku
Tokyo, Japan

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ABSTRACT

Seasonal changes in the incidence of Rice Yellow Orange (Tungro group) virus disease in Thailand, in relation to population and rate of viruliferous individuals of the vector, *Nephotettix virescens*, were studied in field experiments. The disease incidence fairly coincided with population trends of the vector. Infection was found to occur in the nursery as well as in the paddy field. Among several weeds, only wild rice, *Oryza rufipogon*, proved to be an alternate host of the virus. Latent period, *i.e.* the time needed for inoculated plants to become infectious, was found to be as short as 2 days. This, in combination with the nonpersistent nature of the virus transmission, may account for the very high incidences of the disease caused by lower population of the vector, as compared with other rice virus diseases in the temperate zone. The results of insecticide application trials, damage analysis, experiments on the effect of temperature on growth of the vector, the effect of moulting of the vector on virus retention, etc. are also described and discussed.

CONTENTS

I. Introduction

Virus diseases of rice plants, along with diseases caused by mycoplasma-like organism (MLO), are distributed all over Southeast Asia, and cause severe damages. They are Transitory Yellowing, Grassy Stunt, Orange Leaf, Tungro group and Yellow Dwarf. Transitory Yellowing is limited to Taiwan, while the other 4 diseases show wide distributions in South and Southeast Asia. Tungro group includes Tungro in the Philippines and India, Penyakit Merah in Malaysia, Mentek and Penyakit Haban in Indonesia, Yellow Orange Leaf in Thailand (Ou *et al.* 1969).

In Thailand, 4 kinds of virus and MLO diseases have been found: Yellow Orange Leaf, Orange Leaf, Yellow Dwarf, and Grassy Stunt. Among these diseases, Rice Yellow Orange Leaf Virus disease is most prevalent and is one of the most important diseases of rice.

Rice Yellow Orange Leaf Virus disease in Thailand was severe in 1967, 1968, 1969, and 1970, causing serious damages throughout the country. Especially around Bangkok in the Central Plain of Thailand, the rice marketing was profoundly impaired.

The present research was conducted during the period from April 1969 to March 1972, as a joint endeavour between Plant Pathology Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand, and Tropical Agriculture Research Center, Ministry of Agriculture and Forestry, Japan, for the purpose of elucidating field epidemiology of this disease.

II. Acknowledgement

We wish to express our cordial thanks to Dr. Sala Dasananda, former Director General of Rice Department, Ministry of Agriculture, Thailand, Dr. Bhakdi Lusanandana, Director General, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand, Dr. Prakob Kanjanasoon, Deputy Director General, Dr. Sombhot Suwanawong, Deputy Director General, Dr. Tanongchit Wongsiri, Dr. Piya Giatgong, Dr. Kovit Kovitvadhi, Dr. Adul Worawisittumrong, Dr. Dara Buangsuwon, Dr. Paibool Trachoo, and all the members of the former Rice Department, for their proper management and guidance, and also for furnishing laboratory and field facilities.

The writers also wish to express their cordial thanks to Dr. Insee Chansathit, Chairman of the National Research Council of Thailand, Dr. Pradisth Cheosakul, former Secretary General of the Council, Dr. Boonthom Dhamcharee, former Division Chief of Compilation and Coordination of the Council, and the staff members of the Council, for their friendly advice and coordination of the work.

Thanks are also due to Dr. Noboru Yamada, former Director, Tropical Agriculture Research Center, Ministry of Agriculture and Forestry, Japan, Dr. Kan-ichi Murakami, Director of the Center, and all the staff members of the Center; and also to Dr. Jisuke Takahashi, former FAO expert, Dr. Hidetoshi Matsuo, FAO, the United Nations, Dr. Hiroshi Fujii, Dr. Akio Osada, and Dr. Mitsuo Yoshimeki, former Colombo Plan experts, for their critical advice and guidance.

We are also deeply indebted to Dr. A.L. Taylor, former Project Manager of Rice Protection Research and Training Program in Thailand, FAO, the United Nations, for his critical reading of the original manuscript of the report submitted to Thai Government, and also to

Dr. T.T. Iida, former Director of Institute for Plant Virus Research, Ministry of Agriculture and Forestry, Japan, for his discussion and critical reading of manuscript of the present report.

III. Outline of research history of Rice Yellow Orange Leaf Virus

Rice Yellow Orange Leaf Virus (YOLV) was first reported by Luansark Wathanakul at the 4th National Conference on Agriculture and Biology held at Kasetsart University, Thailand, on January 27—29, 1965, the report being mimeographed in Thai on 2 pages. The disease was found at Bangkhen, Bangkok, and its most conspicuous symptom was on leaves, color of which changed into yellowish orange (Wathanakul 1965). In the same year, the virus was successfully transmitted by *Nephotettix* sp. (Wathanakul *et al.* 1965). The damaged area in 1965 was 16,000 ha (King 1966). Varietal resistance was also reported (Breeding Division 1966).

H.A. Lamey, FAO expert, stayed in Bangkok from May 10, 1966 until December 31, 1967. Basic studies were done during his stay with his counterparts, L. Wathanakul, P. Surin, and S. Disthaporn, and the results were presented in their final report (Lamey 1967). The major results were as follows:

1) *Nephotettix virescens* was proved to be the principal vector, and the virus was semi-persistent. *N. nigropictus* transmitted the virus once. Not mechanically transmissible.
2) "Shock phase" symptom appeared 10—13 days after inoculation, which turns into "Masked phase" 18 days after inoculation.
3) Phosphate application slightly suppressed the symptom expression.
4) The symptom on rice in paddy field was most conspicuous 2—3 weeks after transplanting.
5) The damaged area in 1966 was 660,000 ha. Yield decrease by YOLV in pot tests was 60—70% in Taichung Native 1 and 40% in Leuang Tawng.
6) Varieties and lines Peta, Sigadis, IR 5-47-2, and IR 11-452-1-1 were resistant.
7) Diagnosis by infra-red ray photography was possible.
8) Application of Sevin was effective to control the disease.

Partial results of these studies were also reported in preliminary papers and others (Lamey *et al.* 1966, 1967a, Wathanakul *et al.* 1967a,b,c, 1968, 1969 Wathanakul 1969, Annual Research Report, Rice Dept., 1966, 1967).

The following results were also obtained in 1966 and 1967. 1) The acquisition and inoculation thresholds in *N. virescens* were 20 and 15 minutes, respectively. 2) Nymphs transmitted the virus more readily than adults. 3) Adults retained the virus for 6 days. 4) Wild rice was susceptible to the virus (Wathanakul *et al.* 1967c, Annual Research Report, Rice Dept. 1966, 1967).

In 1968, the following results were obtained. 1) *Recilia dorsalis* transmitted the virus. 2) Mealy bugs also transmitted the virus. 3) Viruliferous vector population was low in May and June, and high in July, August, and September. 4) Resistant lines were selected as a part of the program for breeding resistant varieties (Wathanakul 1969, mimeographed subscript for Annual Research Report 1968).

In 1969, highly resistant varieties were bred and named RD-1 and RD-3. These were hybrids between Leuang Tawng and IR-8, and retained the characters of Thai rice quality from the former and YOLV resistance from the latter. Also, RD-2, a glutinous rice variety, was bred from a hybrid between Gam Pai 15 and Taichung Native 1, and proved to be

moderately resistant to YOLV (Jackson *et al.* 1969, Uchiyama 1970). In the process of breeding of these varieties, L. Wathanakul and others joined the selection tests on YOLV resistance. Later, YOLV resistance genes from Peta and Pankhari 203 were found to be dominant (Chantarasnit 1971).

IV. Research results

Part A. Field studies on Rice Yellow Orange Leaf Virus disease

1. Vicissitude of Rice Yellow Orange Leaf Virus disease in Bangkok throughout year

Disease prevalence is influenced by the dispersion speed of the pathogen and also by host conditions, especially the age of the host. These considerations suggest that the disease vicissitude throughout the year should be observed under a more or less constant host age. This may be done by successive cultivation of the host.

In April 1969, Akio Osada, Colombo Plan Expert for rice physiology, planned to analyze high yield factors by year-round cultivations of 5 promising rice varieties and lines under the project "Growth habits of rice planted at one month intervals throughout year" (Osada 1972). We requested permission to make observations on disease occurrences in this experimental field, and the vicissitude of Rice Yellow Orange Leaf Virus (YOLV) disease was surveyed.

Materials and Methods

1) Cultivation: The experimental field was located at Bangkhen, Bangkok. Rice varieties planted were as follows:

RD-2 (Gam Pai/2 × Taichung Native 1)
IR-8 (Peta × DgWg)
C_4-63 (Peta × BPI-76)
BKN-17-3 (Leuang Tawng × IR-8)
RD-1 (Leuang Tawng × IR-8)

These varieties and lines were sown in nursery bed on the 1st day of every month and transplanted on the 21st of the same month. In the paddy field, 128 (8×16) hills of each variety or line were planted at spacing of 25 cm in 2×4 m plots.

2) Disease and insect control: Malathion, BHC, or Sevin was sprayed once a week according to the wide-area control schedule of the experimental field management. No fungicide or bacteriocide was sprayed.

3) Methods of survey: YOLV-diseased hills as mentioned in this chapter are the hills which showed symptoms, and do not include masked hills. In the disease occurrence survey, all the hills planted were observed once a week and the percentage of diseased hills was calculated. The number of leafhoppers on 20 hills was also surveyed once a week.

Experimental Results

1) Seasonal changes of YOLV-disease incidence.

YOLV disease was not observed on any rice varieties and lines transplanted in May,

1969, but occurred severely on plants transplanted in June. On plants transplanted in July, August and September, the disease was also observed, but was not so severe as in June planting. As the cultivating season advanced, the percentage of diseased hills decreased and the symptoms also became milder. Plants transplanted in October and December showed very low percentages of diseased hills and only vague symptoms. No disease was observed on plants transplanted in November 1969, January, February, March, April, and May 1970.

The highest percentage of diseased hills during every cultivating season is shown in Table 1. Differences in varietal resistance were observed. RD-1 was the most resistant, with only 4 hills infected throughout the whole experiment. The next was C_4-63. IR-8 and BKN-17-3 showed almost the same level of moderate resistance: the percentage of diseased hills in these two were slightly higher than in C_4-63. The percentage of diseased hills of the 4 varieties and lines mentioned above were not more than 4%. RD-2 was the most susceptible among the varieties and lines tested. The percentage of diseased hills on RD-2 transplanted in June was 28.9%.

Table 1. Seasonal changes of YOLV-disease incidence in the cultivating seasons of 1969 and 1970

Cultivating season	Highest percentage values of YOLV-diseased hills (%)				
	RD-2	IR-8	BKN-17-3	C_4-63	RD-1
Transplanted in					
May, 1969	0	0	0	0	0
June	28.9	3.1	3.9	1.6	1.6
July	10.9	3.9	2.4	0.8	1.6
August	5.2	0.8	3.5	0.8	0
September	7.0	1.6	0	1.6	0
October	2.3	0.8	0.8	0	0
November	0	0	0	0	0
December	2.3	0	0	0	0
January, 1970	0	0	0	0	0
February	0	0	0	0	0
March	0	0	0	0	0
April	0	0	0	0	0
May	0	0	0	0	0

The date of the first occurrence of YOLV disease in each cultivating season is shown in Table 2. During the period from the 8th to 13th day of the next month after transplanting (about 20 days after transplanting), the disease was first observed in 12 out of 21 cases. In other 4 cases, symptoms were first seen during the period from the 15th to 16th day, and in the remaining 5 cases it occurred later than the 18th day. In the first mentioned 12 cases, the percentage of diseased hills ranged between 28.9 and 1.6%, and in the other 9 cases it was lower than 3.1%.

The highest percentage of diseased hills was observed 1 to 3 weeks after the appearance of symptom, the diseased hills thereafter gradually recovered. At the booting stage it was almost impossible to distinguish diseased hills from healthy ones. This tendency was same in every season and in every variety and line tested. The seasonal change of disease incidence on variety RD-2 is shown in Fig. 1.

Table 2. Time of appearace of YOLV-disease in 1969 and 1970

Cultivating season	Date of YOLV- disease outbreak				
	RD-2	IR-8	BKN-17-3	C_4-63	RD-1
Transplanted in					
May, 1969	—	—	—	—	—
June	July 11	July 16	July 11	July 16	July 11
July	Aug. 13	Aug. 13	Aug. 20	Aug. 20	Aug. 8
August	Sep. 11	Oct. 9	Sep. 11	Sep. 18	—
September	Oct. 9	Oct. 9	—	Oct. 15	—
October	Nov. 10	Nov. 10	Nov. 19	—	—
November	—	—	—	—	—
December	Jan. 15	—	—	—	—
Januray to May, 1970*	—	—	—	—	—

—: No disease was observed.
*: Transplanted in January, February, March, April, and May, 1970.

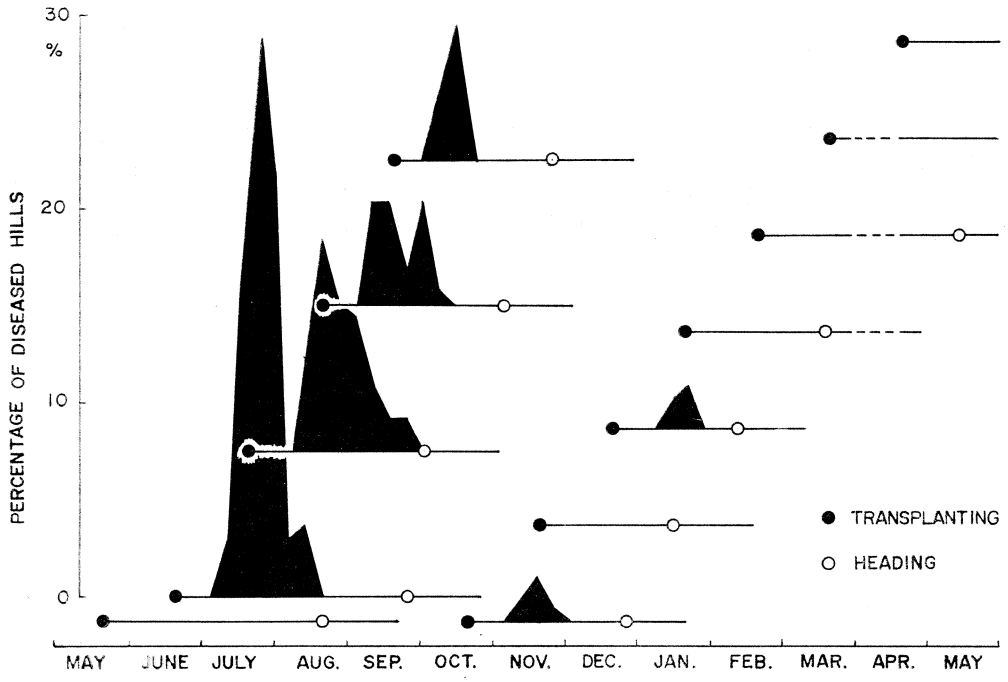

Fig. 1. Seasonal change of Rice Yellow Orange Leaf Virus disease on rice variety RD-2

2) Field population of leafhoppers.

The population of leafhoppers was observed on 20 hills once a week in each field, and the results are shown in Table 3. *Nephotettix* spp. shown in this table include both *N. virescens* and *N. nigropictus*, but the latter was only rarely observed.

Although no *Nephotettix* was observed until the middle of June, many *Nephotettix* were

Table 3. Population of leafhoppers in 1969 and 1970

Cultivating season	Total number of leafhoppers during weekly observations on 20 hills		
	Nephotettix spp.		*Recilia dorsalis*
	Adult	Nymph	Adult
Transplanted in			
May, 1969	28	2	0
June	16	2	7
July	1	19	3
August	0	0	20
September	1	0	1
October	0	0	3
November	2	0	6
December	1	0	1
January, 1970	0	0	6
February	0	0	3
March	0	0	60
April	2	0	21
May	2	0	10

Remarks: Most of *Nephotettix* spp. was *N. virescens*.

observed at the end of this month on rice plants transplanted in May. These plants were already at the stage of internode elongation. On plants transplanted in June, a lot of *Nephotettix* were observed on July 4, two weeks after transplanting. On these plants YOLV disease symptoms were seen on July 11, a week after the leafhopper incidence. On plants

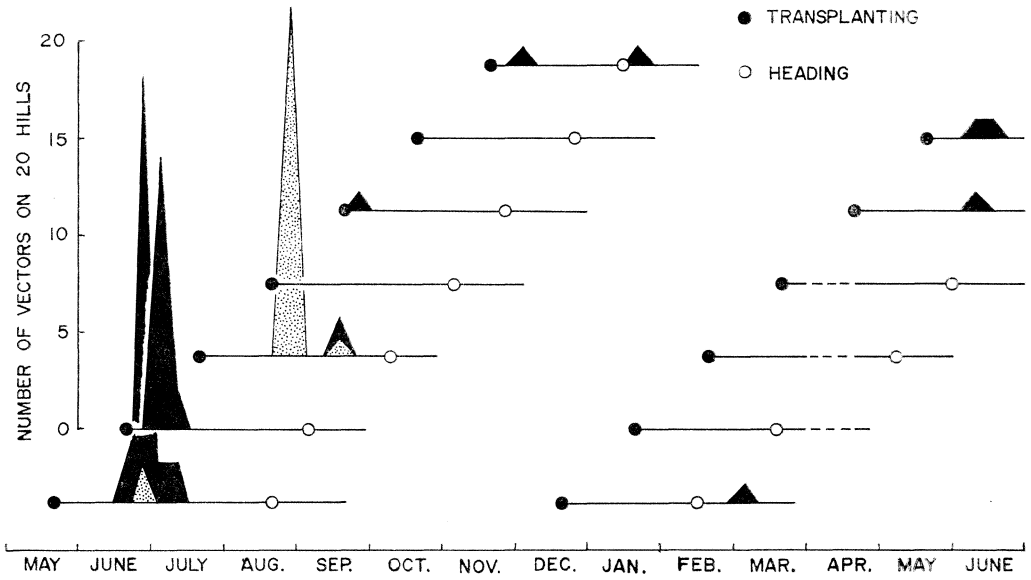

Fig. 2. Seasonal change of *Nephotettix* population in paddy field

transplanted in July, *Nephotettix* nymphs were observed on August 27, about 1 month after transplanting and 2 weeks after the onset of the disease. As the nymphs appeared to be at around the 3rd instar, adults should have been in the field before the disease appearance. On plants transplanted after August, only a few *Nephotettix* were observed. Population of *Nephotettix* spp. in each field is shown in Fig. 2. In addition to *Nephotettix* spp. other leafhoppers were also observed; *Recilia dorsalis* most frequently, *Mahunia grandis*, *Tettigella spectra*, and *Thaia oryzivora* occasionally.

2. Seasonal changes of viruliferous vector population in nursery beds cultivated serially throughout year

In the Central Plain of Thailand, the vector of Rice Yellow Orange Leaf Virus (YOLV), *Nephotettix virescens*, generally diminishes in the dry season, and migrates into paddy fields in the rainy season. Viruliferous vector population in the field was surveyed for several years, and was reported to be low at the beginning of rainy season but become higher later (Wathanakul 1969).

The present chapter deals with the seasonal changes of viruliferous vector population in nursery beds cultivated serially throughout year. The experiment was started in July, 1969, and ended in October, 1970.

Materials and Methods

Rice variety Leuang Tawng was used in the nursery beds throughout year, because this variety is susceptible to YOLV and is non-photosensitive. Each nursery bed was 1.5 m in width and 8 m in length, and was serially cultivated close to each other throughout the year.

To survey the percentage of viruliferous vectors, the vectors were collected from the nursery beds by a suction catcher (D-VAC), and they were individually fed on rice seedlings of Taichung Native 1 variety for 1 day, one vector per seedling in a test tube. The seedlings thus inoculated were transplanted in pots in a screen-house, and kept under observation until symptom appearance. One hundred to 150 vectors were tested once a week.

Experimental Results

When the nursery beds were serially cultivated close to each other throughout year, the vectors, *Nephotettix virescens*, were found even in the dry season. The population in February to May, however, was very low as shown in Table 4, by the number of vectors tested. Despite attempts to collect at least 100 individuals, sufficient number could not be secured. In other months, sufficient number of vectors could be collected in most cases.

Seasonal changes in the percentage of viruliferous vectors are shown in Table 4 and Fig. 3. Five day averages of daily temperature in Bangkok (Division of Climatology, Thailand, 1969, 1970) (in cases of the last interval of every month, the remaining days were lumped), are also shown at the top of Fig. 3.

In the period from September 9 to November 17, the percentage of viruliferous vectors (VV%) was as high as 39.3—64.5%, and was almost constant, despite change of the nursery bed from No. II to No. III during the period. The average temperature (AV Temp.) remained at 27—29°C in September and October, the second half of rainy season; 26—27°C in November, the beginning of dry season.

On November 25, VV% suddenly decreased and remained below 10% until December

Table 4. Percentages of YOLV viruliferous vectors in nursery beds cultivated serially throughout year

Date of survey		Number of vectors tested	Number of viruliferous vectors	Percentage of viruliferous vectors (%)	Nursery bed No.
1969					
July	14	143	0	0	I
	21	100	1	1.0	I
	28	150	2	1.3	I
Aug.	4	150	7	4.7	I
	11	150	9	6.0	II
	18	150	6	4.0	II
	25	150	4	2.7	II
Sep.	1	147	12	8.2	II
	9	150	69	46.0	II
	15	150	73	48.7	II
	22	149	96	64.5	II
	29	150	64	42.7	II
Oct.	6	147	62	42.2	III
	13	144	57	39.6	III
	20	149	65	43.6	II
	27	148	84	56.8	III
Nov.	3	149	67	45.0	III
	11	150	59	39.3	III
	17	150	84	56.0	III
	25	40	3	8	IV
Dec.	1	37	0	0	IV
	8	100	3	3.0	IV
	16	157	2	1.3	IV
	23	45	2	4	IV
1970	30	59	5	8	IV
Jan.	6	146	8	5.5	V
	13	159	25	15.7	V
	20	148	25	16.9	V
	27	44	13	30	V
Feb.	3	17	5	—**	V
	10	39	6	15	V
	17	55	22	40	V
	24	8	0	—	VI
Mar.	3	33	1	3	VI
	10	23	1*	—	VI
	16	20	0	—	VI
	26	14	1	—	VI
Apr.	7	38	1	3	VII
	14	20	1*	—	VII
	21	9	0	—	VII
	27	20	2*	—	VII
May	4	6	1	—	VIII
	11	19	0	—	VIII
	18	15	1*	—	VIII
	26	25	1*	—	VIII

Table 4. Continued

Date of survey	Number of vectors tested	Number of viruliferous vectors	Percentage of viruliferous vectors	Nursery bed No.
June 1	45	0	0	IX
10	71	1*	1	IX
15	95	1	1	IX
17	77	0	0	IX
22	20	0	—	IX
28	122	1	0.8	X
July 7	114	1	0.9	X
14	144	6	4.2	X
21	49	7	14	X
29	40	7	18	XI
Aug. 4	83	6	7	XI
11	130	21	16.2	XI
18	107	32	29.9	XI
25	95	13	14	XI
Sep. 15	91	26	29	XII
28	110	62	56.4	XII
Oct. 5	113	50	44.2	XII

Remarks: In the tests after June 17, 1970, the vectors used were definitely *N. virescens*, but before this date, there are posibilities of some contamination with *N. nigropictus*.
*: Symptom on rice was not clear.
**: Mark denotes that percentage was not calculated as the number of vectors tested was less than 30.

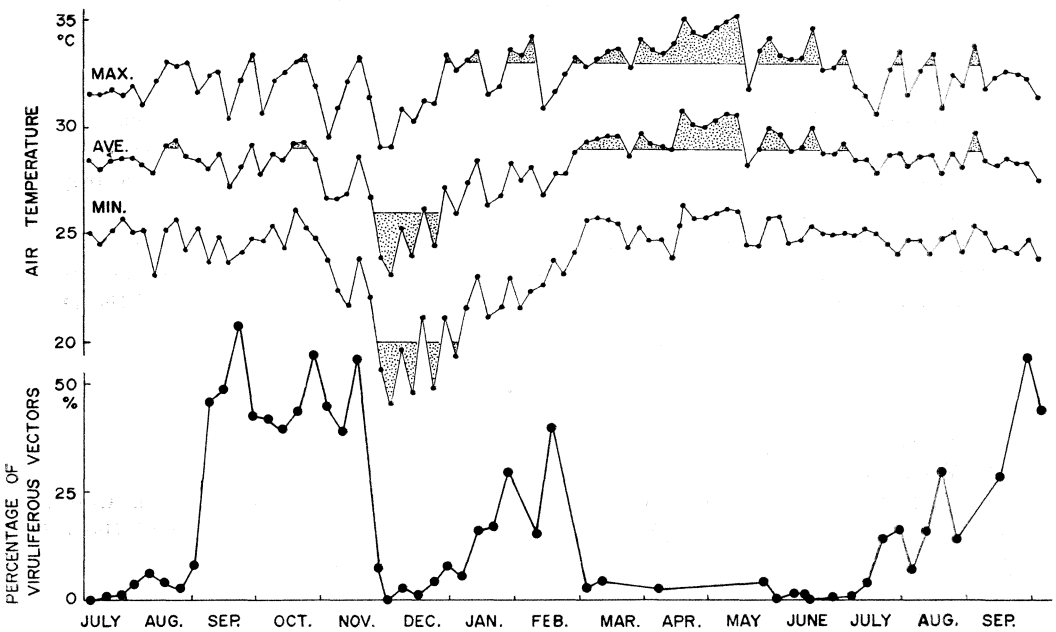

Fig. 3. Seasonal change in the percentage of viruliferous vectors and air temperature

30 in the nursery bed No. IV. AV Temp. also suddenly dropped at the end of November and remained at 23—26°C during this period. Minimum temperature was usually lower than 20 °C.

In the period from January 6 to February 17, in the nursery bed No. V, VV% gradually increased to about 40% (22 out of 55 vectors were viruliferous). AV Temp. in this period (26—29°C) was about the same as in the rainy season.

From February 24 to May 26, in the nursery beds No. VI, VII, and VIII, the vector population was low, and only 38 vectors could be collected for the test. Viruliferous vectors were not more than 2 in each survey, and VV% was considered to be a few percent, though the result was not accurate due to the low vector population. AV Temp. was 29—31°C and the maximum temperature rose to 33—35°C. This period was the dry and hot season.

In the period from June 1 to 22, in the nursery bed No. IX, the vector population gradually increased, but VV% still remained at a few percent. The temperature slightly dropped in accordance with rainfall, but AV Temp. was still high (29—30°C).

From June 28 to August 25, the first half of rainy season, in the nursery beds No. X and XI, the vector population was high and VV% gradually increased. The values of VV% obtained were higher than those of the previous year in the nursery bed No. I. These higher values are considered to be caused by the increase in disease sources as the result of serial and continuous nursery bed cultivation. AV Temp. declined to 28—29°C, which was the ordinary temperature in the rainy season.

In September and October, in the nursery bed No. XII, VV% reached the highest peak. AV Temp. remained at 28—29°C the same as in the previous year in the nursery beds No. II and III.

3. Time of infection with Rice Yellow Orange Leaf Virus in nursery beds and paddy fields in Bangkok

In farmers' paddy fields it was frequently observed that almost all plants were infected by Rice Yellow Orange Leaf Virus (YOLV) disease, showing yellowing and slight stunting. Also in nursery beds, almost all seedlings were sometimes observed to be infected. There were rare cases where diseased hills scattered among healthy ones, apparently the beginning stage of disease spread.

From these observations, the causal virus seemed to be transmitted rather uniformly in nursery beds and spread rapidly in paddy fields. In order to determine the infection time, disease occurrences were closely traced in nursery beds and paddy fields of the Department of Agriculture, Bangkhen, Bangkok, and the results were analyzed.

Materials and Methods

1) Experiment I.

a) Variety used: Thai native rice variety Leuang Tawng.

b) Nursery bed: Three beds each 1.5×8 m in size were prepared. Seeds were sown on June 26, 1970, and fertilizer (Ammonium-sulfate, N=21%) was applied at the rate of 100 g per bed. The beds were separately used for the following purposes:

Bed A: To assay vector population.
Bed B: To survey percentage of viruliferous vectors.
Bed C: To grow seedlings to be transplanted in paddy field.

c) **Paddy field**: The seedlings grown in Bed C were transplanted in the paddy field at different times, 25, 36, and 45 days after sowing, with spacing of 25×12.5 cm, one plant per hill. At every transplanting time, two kinds of plots, open plots and screen-cage plots, were provided, the plots being separated by small dikes.

Open plots: Under natural conditions. 500 hills at every transplanting time. No insecticide sprayed.

Screen-cage plots: Covered by 2 screen-cages, each 250×90×90 cm in size and covered by plastic screen. Also protected from vectors by subsequent insecticide spraying. 160 to 176 hills at every transplanting time.

Fertilizer Ammophos (N=16%, P=20%) was applied to both plots at the rate of 150 kg per ha about 1 week after transplanting.

d) **Vector population**: The population of *Nephotettix virescens* and *N. nigropictus* was assayed by sweeping 20 times with an insect net in the case of nursery beds, or by counting the number of vectors on 20 hills in the case of paddy fields. These surveys on vector population were kindly done by the entomologist Mr. H. Inoue.

e) **Percentage of viruliferous vectors**: This survey was done only in the nursery beds. The collected vectors, *N. virescens* were fed for 1 day on 10-day-old seedlings of Taichung Native 1 in test tubes, one vector per seedling. The seedlings were then transplanted in pots in a screen-house and symptom appearance was observed.

f) **Percentage of diseased hills in nursery beds**: The total number of seedlings in nursery beds was estimated from seedling density which was approximately 3,000 per square meter. From the number of diseased seedlings observed and the total number of seedlings thus estimated, the percentage of diseased hills was calculated.

g) **Percentage of diseased hills in paddy field**: Diseased hills were marked on a map of the experimental field which showed every hill. The diseased hills include both the hills showing clear symptoms and the hills which recovered after once showing clear symptoms. In order to get the total number of hills, missing hills due to various causes, *e.g.*, flating away, Kresek-disease, were also marked on the map. The percentage of diseased hills in the paddy field was calculated from these data.

2) Experiment II.

This is a replication of Experiment I. The seeds were sown on August 20, 1970, and transplanted 25 and 35 days after sowing. Other procedures were the same as in Experiment I.

Experimental Results

1) Experiment I.

In the nursery bed where seeds were sown on June 26, 5 diseased seedlings per 12 m² were observed 25 days after sowing, and 26 seedlings after 36 days. The percentage of dis-

Table 5. YOLV-disease occurrence in nursery bed in Experiment I (Sowing on June 26, 1970)

Date of survey	Days after sowing	Number of diseased hills in 12 m²	Percentage of diseased hills* (%)
July 12	25	5	0.01
Aug. 1	36	26	0.07
Aug. 10	45	Many hills showed dubious symptoms	

*: From estimated seedling density, 3,000/m².

eased hills was calculated to be 0.01% and 0.07% respectively. After 45 days, many seedlings showed dubious symptoms. The results are shown in Table 5.

Table 6. Number of hills used for YOLV disease survey in paddy fields in Experiment I (Sowing on June 26, 1970)

Duration in nursery bed	Date of trans-planting	Plot*	Number of hills				
			Trans-planted	Missing by floating away	Damaged by Kresek-disease	Killed for unknown cause	Used for YOLV-disease survey
25 days	July 21	OPE	500	14	5	3	478
		SCR	160	6	3	1	150
36 days	Aug. 1	POE	500	2	2	11	485
		SCR	176	3	0	3	170
45 days	Aug. 10	OPE	500	4	0	6	490
		SCR	176	4	0	0	172

*OPE: Open plot, SCR: Screen-cage plot.

Table 7. YOLV-disease occurrence in paddy fields in Experiment I (Sowing on June 26, 1970)

Date of survey	Days after trans-planting	Open plot		Screen-cage plot	
		Number of diseased hills	Percentage of diseased hills (%)	Number of diseased hills	Percentage of diseased hills (%)
Transplanted on July 12 (25 days after sowing)					
Aug. 2	12	4	0.8	0	0
5	15	11	2.2	0	0
8	18	20	4.2	0	0
13	23	40	8.4	0	0
17	27	86	18.0	0	0
22	32	130	27.2	0	0
27	37	194	40.5	0	0
Sep. 10	51	362	65.2	0	0
Transplanted on August 1 (36 days after sowing)					
Aug. 13	12	9	1.9	8	4.7
15	14	15	3.1	9	5.3
17	16	17	3.5	10	5.9
22	21	21	4.3	10	5.9
27	26	54	11.1	10	5.9
Sep. 2	32	76	15.7	10	5.9
10	40	165	34.0	10	5.9
Transplanted on August 10 (45 days after sowing)					
Aug. 23	13	219	44.7	116	68.2
27	17	292	59.6	131	77.0
Sep. 2	23	328	66.9	135	79.4
10	31	438	89.4	135	79.4

The seedlings were transplanted to the paddy field 25, 36, and 45 days after sowing from the nursery bed, as mentioned above. As some hills disappeared at the rooting stage just after transplanting, because of floating away, Kresek-disease, and other unknown causes, those hills were excluded from the survey. The number of hills used for the survey is shown in Table 6.

In the paddy field, increase of diseased hills was observed in both screen-cage plot and open plot. The results are shown in Table 7. In the case of plants transplanted 25 days

Table 8. Percentage of viruliferous vectors in nursery bed in Experiment I (Sowing on June 26, 1970)

Date of survey	Days after sowing	Adult		Nymph	
		Number of vectors tested	Percentage of viruliferous vectors (%)	Number of vectors tested	Percentage of viruliferous vectors (%)
July 29	33	10	10	30	20
Aug. 4	39	60	10	23	0
11	46	130	16	—	—
18	53	94	31	13	23
25	60	68	10	27	22

Table 9. Vector population in nursery bed and paddy fields in Experiment I (Sowing on June 26, 1970)

Date of survey	Days after sowing or trans-planting	Number of vectors					
		N. virescens			*N. nigropictus*		
		Male adult	Female adult	Nymph	Male adult	Female adult	Nymph
Nursery bed (Sown on June 26)*							
July 6	11	24	10	0	1	0	0
24	29	20	23	52	0	1	0
Aug. 1	37	32	24	101	6	6	0
Open plot in paddy field**							
(Transplanted on July 21, 25 days after sowing)							
July 24	3	1	0	0	0	0	0
29	8	0	0	0	0	0	0
Aug. 4	14	0	0	0	0	0	0
13	23	1	0	5	0	0	0
27	37	1	1	2	0	0	0
(Transplanted on August 1, 36 days after sowing)							
Aug. 4	3	0	0	0	0	0	0
13	12	0	0	0	0	0	0
27	26	0	2	7	0	0	0
(Transplanted on August 10, 45 days after sowing)							
Aug. 27	17	1	1	1	0	0	0
Sep. 11	32	1	0	3	1	0	0

*: Sweeping 20 times.
**: Observation on 20 hills.

after sowing, no diseased hills were found in the screen-cage plot where no infection occurred in the paddy field, while the disease increased gradually in the open plot. This result shows that infection occurred only in the paddy field. In the case of plants transplanted 36 days after sowing, the percentage of diseased hills in the screen-cage plot was 5.9% and the percentage in the open plot increased gradually to more than 5.9%. This shows that 5.9% of hills were infected in the nursery bed and infection also occurred in the paddy field. In the case of plants transplanted 45 days after sowing, the percentage of diseased hills in the screen-cage plot reached 79.4% and that in open plot 89.4%. In this case main infection is considered to have occurred in the nursery bed.

Changes in the percentage of viruliferous vectors in the nursery bed are shown in Table 8. One viruliferous adult was found out of 10, and 6 nymphs out of 30 after 33 days from sowing; a higher rate was observed later.

The vector population in the nursery bed was not so high and that in the paddy field was very low, as shown in Table 9.

2) Experiment II.

In the nursery bed where seeds were sown on August 20, 14 diseased seedlings per 12 m^2 were observed 21 days after sowing, and 28 diseased seedlings after 25 days. After 38 days, 1,060 seedlings per 12 m^2 were diseased, and many other seedlings showed dubious symptoms. The percentages of diseased hills estimated by the same method as in Experiment I, were 0.04%, 0.08%, and 3%, respectively, as shown in Table 10.

Table 10. YOLV-disease occurrence in nursery bed in Experiment I (Sowing on August 20, 1970)

Date of survey	Days after sowing	Number of diseased hills in 12 m^2	Percentage of diseased hills* (%)
Sep. 10	21	14	0.04
Sep. 14	25	28	0.08
Sep. 27	38	1,060**	3

* From estimated seedling density, 3,000/m^2.
** Besides these, many seedlings showed dubious symptoms.

Table 11. Number of hills used for YOLV-disease survey in paddy fields in Experiment II (Sowing on August 20, 1970)

Duration in nursery bed	Date of trans-planting	Plot*	Number of hills				
			Trans-planted	Missing by floating away	Damaged by Kresek-disease	Killed for unknown cause	Used for YOLV-disease survey
25 days	Sep. 14	OPE	500	53	94	6	347
		SCR	176	27	40	0	109
35 days	Sep. 24	OPE	500		192**		308
		SCR	176		44**		132

* OPE: Open plot, SCR: Screen-cage plot.
** Total number of hills missing by floating away, damaged by Kresek-disease and killed by unknown reason.

Seedlings were transplanted in the paddy field 25 and 35 days after sowing. The number of hills used for the YOLV-disease survey is shown in Table 11. Many more hills disappeared by floating away, Kresek-disease, or other unknown causes than in Experiment I.

The increase of YOLV disease in the paddy field is shown in Table 12. In the plants transplanted 25 days after sowing, 24.8% of the hills in the screen-cage plot were observed to be infected, while diseased hills in the open plot rapidly increased and reached 80.4% at the end of the observation. This result shows that 24.8% infection occurred in the nursery bed, and additional infection in the paddy field resulted in 80.4% total diseased hills within 27 days after transplanting. In the plants transplanted 35 days after sowing, where 93.2% of the hills in the screen-cage plot and also 94.5% in the open plot were diseased, almost all infection may have occurred in the nursery bed.

Table 12. YOLV-disease occurrence in paddy fields in Experiment II (Sowing on August 20, 1970)

Date of survey	Days after transplanting	Open plot		Screen-cage plot	
		Number of diseased hills	Percentage of diseased hills (%)	Number of diseased hills	Percentage of diseased hills (%)
Transplanted on September 14 (25 days after sowing)					
Sep. 27	13	67	19.3	21	19.3
Oct. 1	17	116	33.4	25	23.0
7	23	197	56.8	26	23.8
11	27	279	80.4	27	24.8
Transplanted on September 24 (35 days after sowing)					
Oct. 7	13	165	53.6	114	86.4
11	17	286	92.9	122	92.5
17	23	291	94.5	123	93.2

The percentage of viruliferous vectors in the nursery bed was already 30% in adults and 25% in nymphs 26 days after sowing, and 56% in adults 39 days after sowing. These figures were remarkably higher than those in Experiment I. The results are shown in Table 13.

The vector populations in the nursery bed and paddy field, as shown in Table 14, were not so high and about equal to those in Experiment I.

Table 13. Percentage of viruliferous vectors in nursery bed in Experiment II (Sowing on August 20, 1970)

Date of survey	Days after sowing	Adult		Nymph	
		Number of vectors tested	Percentage of viruliferous vectors (%)	Number of vectors tested	Percentage of viruliferous vectors (%)
Sep. 15	26	71	20	20	25
Sep. 28	39	110	56	—	—
Oct. 5	46	80	49	33	33

Table 14. Vector population in nursery bed and paddy fields in Experiment II (Sowing on August 20, 1970)

Date of survey	Days after sowing or trans-planting	Number of vectors					
		N. virescens			*N. nigropictus*		
		Male adult	Female adult	Nymph	Male adult	Female adult	Nymph
Nursery bed (Sown on August 20)*							
Sep. 1	12	11	8	0	1	2	0
15	27	23	16	35	3	3	0
Open plot in paddy field**							
(Transplanted on September 14, 25 days after sowing)							
Sep. 23	9	0	0	0	0	0	0
30	16	0	0	0	0	0	0
Oct. 10	26	2	0	0	0	0	0
(Transplanted on September 24, 35 days after sowin)							
Sep. 30	6	0	0	0	0	0	0
Oct. 10	16	0	1	0	0	0	0
15	21	1	0	0	1	0	0

* Sweeping times.
** Observation on 20 hills.

Table 15. Temperature in Open plot and Screen-cage plot

Date	Time	Temperature (°C)			Weather
		Open plot	Screen-cage plot	Difference	
Aug. 23	10.30	32.5	31.7	0.8	slightly cloudy
24	—	30.0	29.5	0.5	cloudy
25	10.30	29.9	29.5	0.4	cloudy
25	15.30	31.5	31.0	0.5	slightly cloudy
27	10.30	29.1	28.8	0.3	slightly cloudy
Sep. 2	—	29.7	29.7	0	fine
10	15.00	28.7	28.3	0.4	cloudy

3) Temperature conditions in screen-cage plot and open plot.

Temperatures in the screen-cage plot and in the open plot are shown in Table 15. The temperature in the screen-cage plot was a little lower than in the open plot, although the difference was less than 1°C.

4) Viruliferous vector population in paddy field.

The vector population in the paddy field was not high, for example, the highest population on 20 hills observed was only 9. It is interesting that such a low population of vectors resulted in a heavy infection.

We planned to assess viruliferous vector population also in paddy fields, but the vectors could not be collected from the experimental field. Instead, we collected them from paddy fields adjacent to the experimental field. The percentage of viruliferous vectors is shown

Table 16. Percentage of viruliferous vectors in paddy field

Materials and methods:
Rice variety: Taichung Native 1, at tillering stage.
Date of survey: August 12, 1970.
Area of vector collection: 250 square meters.
Percentage of diseased hills in the paddy field: 98%.
Methof of the test: After collecting the vectors by insect net, they were tested by the methods described in the text.
Results: Adult: 46% were viruliefrous out of 52.
Nymph: 23% were viruliferous out of 22.

in Table 16. Although these figures are not the actual percentage of viruliferous vectors, in the experimental field, it is probable that vectors in the experimental field might also have been highly viruliferous. The virus-transmissibility of vectors in the paddy field may be of the same order as in the nursery bed.

5) Analysis of the disease progress.

For the purpose of comparing the disease progress in the nursery bed and paddy field, the concept of "infection rate" (Plank 1963) was applied. When the percentage of diseased hills is converted into logit, $\log_e (x/(1-x))$, theoretically an S-shaped curve will be replaced by a straight line. Disease progress can then be represented by the slope of the straight line, which is called the "infection rate".

In the values of percentage of diseased hills in the open plots, the values in excess of the percentage of infected hills at the time of transplanting are considered to be caused by the paddy field infection. These are plotted in Fig. 4 and show straight lines. The slopes of these lines were in the range of 0.121 to 0.196, and are shown in Table 17 as the infection rates in the paddy field.

The percentage of diseased hills observed in the nursery bed was unsuitable for calculating the infection rate because the symptoms were very vague. The denominator for percentage calculation was the estimated number, but the values were too small. In calculating the infection rate in the nursery bed, the percentage of infected hills at the time of transplanting should be used. This was given by the maximum percentage of diseased hills in screen-cage plots mentioned above. The infection rates in the nursery bed thus calculated are shown

Table 17. Infection rate of YOLV-disease in nursery bed and paddy field

Experiment No.*	Site of survey	Date of transplanting	Infection rate (r)**	Number of data used for the calculation
I	Nursery bed	—	0.458	2
I	Paddy field	July 21	0.171	7
		Aug. 1	0.121	4
		Aug. 10	0.181	2
II	Nursery bed	—	0.373	2
II	Paddy field	Sep. 14	0.196	4

* Seeds were sown on June 26 in Exp. I, and on August 20 in Exp. II.
** The infection rate in nursery bed was calculated from the data in screen-cage plots, and the rate in paddy fields was from those in open plots.

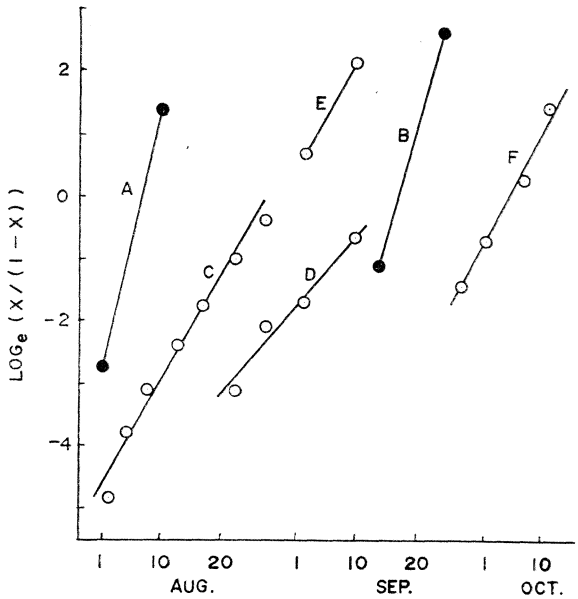

Fig. 4. Infection and disease progress in nursery bed and paddy field

A & B: The infection progress in the nursery bed of Exp. I and II respectively.

C to F: The disease progress in the paddy field, for the plants transplanted 25 days (C), 36 days (D), and 45 days (E) in Exp. I, and 25 days (F) after sowing, in Exp. II.

x: Rate of infected hills (A & B), and of diseased hills (C to F).

also in Table 17, and were 0.458 and 0.373 in Exp. I and II, respectively. If the incubation period is supposed to be equal, though there might be slight differences according to seedling age, the infection rates in the nursery bed and paddy field can be compared. The infection rates in the nursery bed in Exp. I were 2.5 to 3.8 times higher than in the paddy field; in Exp. II 1.9 times higher. Thus disease progress in the nursery bed was distinctly faster than in the paddy field.

4. Insecticide application for the control of Rice Yellow Orange Leaf Virus disease

In Thailand, rice varieties resistant to Rice Yellow Orange Leaf Virus (YOLV) disease have been developed as one of the most important control measures of the disease. Among hybrids between Thai and foreigh varieties, resistant varieties RD-1 and RD-3 were selected, and released to farmers in 1969. These are hybrids between Leuang Tawng and IR-8, and are high yielding and YOLV-resistant (Jackson *et al.* 1969). However, because farmers had to change their cultivating practices and introduce new technics using fertilizers, irriga-

tion water control, *etc.*, these varieties were not rapidly adapted by farmers, and are as yet not widely planted. It is therefore sometimes neccessary to apply insecticides in order to control this disease.

The present chapter deals with determination of suitable time of insecticide application based on observations of disease developments. The insecticide used was Sevin, which had been proved effective for the control of YOLV disease (Lamey 1967).

Materials and Methods

1) Rice variety and insecticide used.

The rice variety Taichung Native 1, which is very susceptible to YOLV-disease, was used. The insecticide used was Sevin Granules which contained 5% of the active ingredient, 1-naphthyl-N-methylcarbamate. At each application time, 40 kg/ha of Sevin Granules were applied.

2) Cultivation.

The field used is located at Bangkhen, Bangkok, and belongs to the Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand.

Germinated seeds were sown on August 1, 1969, in 4 nursery beds, each of which was 1.5×8 m in size. Three among these 4 beds were close to each other, while the other bed was 10 m apart from the 3 beds. The insecticide was applied in the former 3 beds; it was not applied in the 4th bed. All the nursery beds were irrigated throughout the experiment.

In paddy field, 24 plots (8 plots and 3 replications) were separated by dikes. Between plots a space 1 m wide was left uncultivated and filled with water. The plots were 8×13 m in size and the whole paddy field used was 60×60 m square. Fertilizer Ammophos (N=16%, P_2O_5=20%) was applied at the rate of 300 kg/ha in all the plots before transplanting, and no top-dressing was applied later. Just after transplanting, 30 kg/ha of BHC Granules (BHC=6%) was applied to prevent injury by crabs.

The growth of seedlings in the nursery beds was in general good and uniform. The leaf stage and height of seedlings were as follows: 5 days after sowing, 2.3 leaf stage and 4 cm high; 13 days, 5.0 leaf stage and 15 cm high. At the time of transplanting, 23 days after sowing, the seedlings were 6.0 leaf stage and 27.8 cm high, with no secondary tillers.

In the paddy field one plant per hill was transplanted at 25 cm intervals on August 23. The number of hills in each plot was $51 \times 31 = 1{,}581$. The plants reached heading stage on October 13 in Plot H, in which many healthy plants were observed. They were harvested on November 27.

3) Insecticide application.

In 3 of the 4 nursery beds, Sevin Granules were applied on August 8, 13 and 18. In the paddy field, 8 plots were prepared and designated A, B, C, D, E, F, G, and H. In Plot A and B, the seedlings were transplanted from the nursery bed where the insecticide was not applied, and in Plot C, D, E, F, G, and H from the beds where the insecticide was applied. The dates of insecticide application in each plot are shown in Table 18. The insecticide was not applied in Plot A and C of the paddy field. The insecticide was applied 4 times in Plot B and H, and was applied twice at different timings in Plots D, E, F, and G.

4) YOLV disease incidence.

In the paddy field, diseased hills among 496 hills (16×31) in the central part of each plot were counted on September 12, 19, 22, and 26 and October 3. Only those hills showing clear symptoms of YOLV disease were designated as diseased.

On September 15, October 9 and 21, hills which showed dubious symptoms were also counted. These were lumped together with the clearly diseased hills and designated "abnormal" hills.

Table 18. Dates of insecticide application in each plot

Plot	Nursery bed			Paddy field			
	Aug. 8	13	18	Aug. 26	Sep. 2	9	16
A	—	—	—	—	—	—	—
B	—	—	—	*	*	*	*
C	*	*	*	—	—	—	—
D	*	*	*	*	*	—	—
E	*	*	*	—	*	*	—
F	*	*	*	—	—	*	*
G	*	*	*	*	—	*	—
H	*	*	*	*	*	*	*

*: Applied,
— Non-applied.

5) Survey on plant growth and yield.

Height, culm length, and number of panicles were counted on 62 hills in each plot on November 5, when the plants were at the final stage of ripening. For the survey of yield, the 496 hillls in 34 m² of the central part of each plot mentioned above were harvested and threshed immediately. The weight of unhusked grains were measured after drying.

6) Survey on virus-vector population.

In the nursery beds, the vectors were collected by sweeping 20 times with an insect net on August 13, 18, and 20. On August 22, one day before transplanting, the vectors in 1.5× 1.5 m were collected by suction catcher, D-VAC. In the paddy field, the number of vectors on 20 hills in each plot was counted on September 26.

Experimental Results

1) Percentage of diseased hills.

During the nursery period no diseased hills were observed, although observations were made 8 times. In the paddy field, the disease appeared 2 weeks after transplanting. The percentage of diseased hills was surveyed 5 times from September 12 to October 3. In these surveys, significant differences among plots were found in the results obtained on September

Table 19. Percentage of diseased hills on September 26 (34 days after transplanting)

Plot	Percentage of diseased hills (%)				Significant difference from	
	Repl. I	II	III	Average	Plot A	H
A	37.7	32.2	8.1	26.0		**
B	6.5	4.2	13.1	7.9	**	
C	21.0	10.6	9.0	13.5		*
D	8.2	5.7	5.2	6.4	**	
E	7.9	6.9	3.0	5.9	**	
F	24.6	9.2	2.0	11.9	*	*
G	8.2	5.6	9.1	7.6	**	
H	4.3	1.5	2.3	2.7	**	

22 and 26, and October 3. The percentage of diseased hills in Plot A was significantly higher than in Plots B, D, E, F, G, and H. In Plot A, no insecticide was applied, whereas insecticide was applied in the other plots at some stages of the plant growth. This result shows effectiveness of insecticide application in the paddy field. The result of the survey on September 26 is shown in Table 19.

To determine the effect of insecticide application in nursery bed, Plot A was compared with Plot C, and also Plot B with Plot H, because the treatments in these combinations were different in the nursery bed but equal in the paddy field. The percentage of diseased hills on September 26 in Plot A was higher than that in Plot C, and that in Plot B was also higher than that in Plot H. This suggests that the insecticide application in the nursery bed was effective, although the differences were not significant.

Among the plots to which insecticide was applied, the lowest percentage of diseased hills was observed in Plot H, where the insecticide was applied 4 times in the paddy field, but the difference was significant only in one case, *i.e.* between Plot H and Plot F.

Changes in the percentages of diseased hills with the lapse of time are shown in Fig. 5. The percentages of diseased hills in Plots B and H, where the insecticide was applied 4 times in the paddy field, show no increase until September 26. In Plot D, where the insecticide was applied on August 26 and September 2, and also in Plot E, where it was applied on September

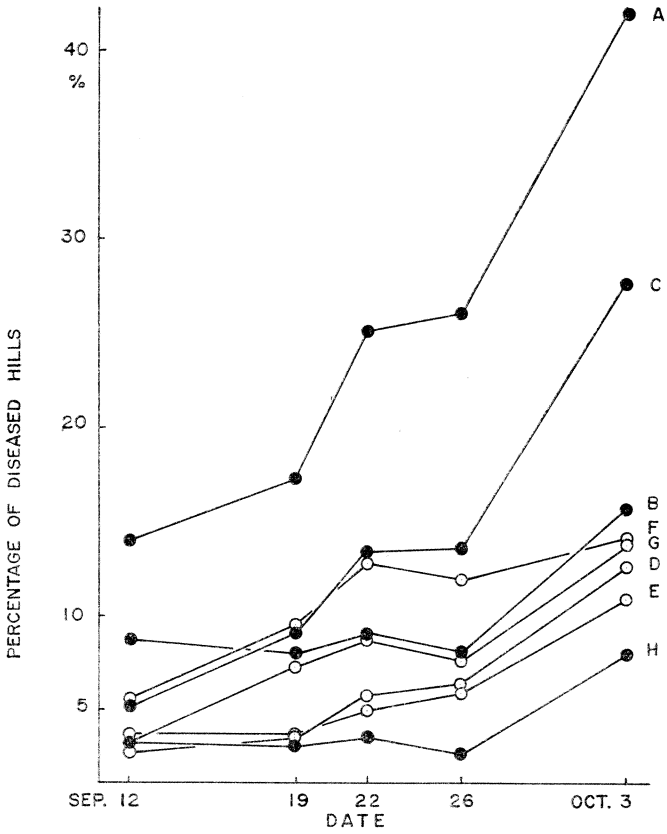

Fig. 5. Effect of Sevin application at different times on the percentage of YOLV-diseased hills

2 and 9, the percentages of diseased hills show no increase or only a slight increase until September 19; later there is a gradual increase. On the other hand, in Plot F, where the insecticide was applied on September 9 and 16, and also in Plot G, where it was applied on August 26 and September 9, there are increases in the percentage of diseased hills until September 19 or 22, but later there are same tendencies of increase as in Plots B and H.

2) Percentage of "abnormal" hills.

The percentages of "abnormal" hills were surveyed 3 times. No significant differences were observed among plots on September 15. In the survey on October 9 and 21, significant differences were observed between Plot A and each of the Plots B, E, F, and H, and also between Plot C and each of the Plots B, F, and H. Among the plots where insecticide was applied, significant differences were observed only between Plot H and Plots D and G. The former was the plot where the insecticide was applied 4 times, while the latter plots recieved the insecticide twice. Among the plots treated twice, no significant differences were observed. The result of the survey on October 9 is shown in Table 20.

Changes in the percentages of abnormal hills with the lapse of time are shown in Fig. 6. The percentages of abnormal hills in Plot A and C, where the insecticide was not applied, show a remarkable increase. In the plots treated twice, abnormal hills increased gradually, while in the plots with 4 applications the percentage increase was the lowest among the plots tested.

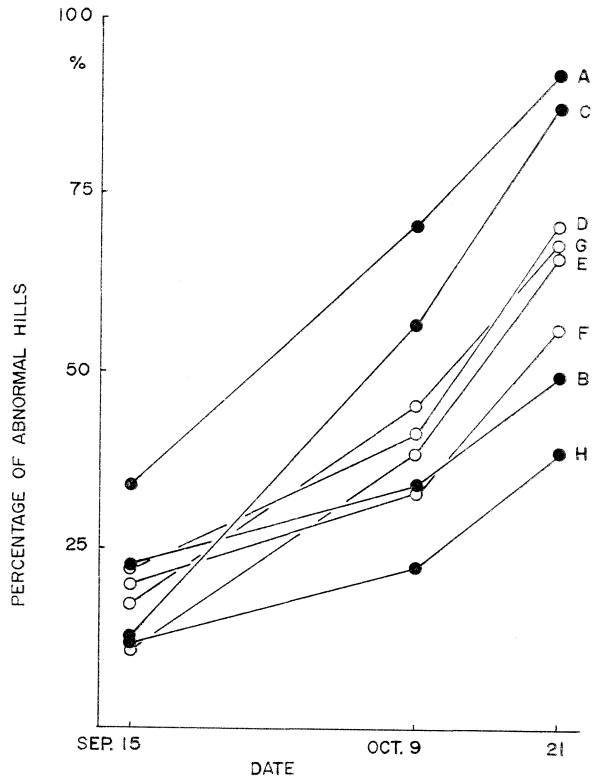

Fig. 6. Effect of Sevin application at different times on the percentage of abnormal hills

Table 20. **Percentage of "abnormal" hills on October 9 (49 days after transplanting)**

Plot	Percentage of abnormal hills (%)				Significant difference from		
	Repl. I	II	III	Average	Plot A	C	H
A	63.4	60.9	87.4	70.6			**
B	17.8	14.3	70.0	34.0	**	*	
C	52.7	47.1	70.8	56.9			**
D	27.8	41.3	56.0	41.7	*		*
E	34.6	41.6	39.8	38.7	**		
F	36.8	23.8	38.3	33.0	**	*	
G	21.4	38.5	77.0	45.6	*		*
H	14.5	3.6	50.4	22.8	**	**	

3) Plant height, culm length and number of panicles at ripening stage.

The plants in Plot H were observed to be the highest; those in Plot B the next highest. These were the plots treated 4 times, and the plants in these plots were very much higher than in the other plots. The plant height in Plot H was significantly different from the other plots, except Plot B. The shortest plant height was observed in Plot A, where the insecticide was not applied both in nursery bed and paddy field; the plant height in Plot C where insecticide was applied only in nursery bed was next shortest. The plant heights in Plot A and C were significantly different from those in the other plots except Plot D and E. The results are shown in Table 21.

The same tendency as the height was observed for culm length. The longest were the plants in Plot H and the next were in Plot B. The shortest were in Plot A and the next were in Plot C. The culm length in Plot H was significantly different from that in Plots A, C, D, E, and G, and that in Plot A was significantly different from that in Plots B and H. The results are shown in Table 22. As for the number of panicles, significant differences were not observed.

4) Yield.

The highest yield was recorded in Plot H, where the insecticide was applied 3 times in the nursery bed and 4 times in the paddy field. The next was in Plot B, where the insecticide was not applied in the nursery bed but applied 4 times in the paddy field. The lowest was

Table 21. **Plant height at ripening stage**

Plot	Height (cm)				Significant difference from			
	Repl. I	II	III	Average	Plot A	B	C	H
A	58.9	58.6	59.2	58.9		**		**
B	78.0	69.8	68.6	72.1	**		**	
C	58.8	65.7	55.2	59.9		**		**
D	67.6	65.5	60.7	64.6		*		**
E	64.4	63.1	60.5	62.7		**		**
F	73.2	69.1	59.3	67.2	*		*	*
G	71.4	68.3	60.2	66.6	*		*	*
H	76.4	81.7	64.7	74.3	**		**	

Table 22. Culm length at ripening stage

Plot	Cuml length (cm)				Significant difference from			
	Repl. I	II	III	Average	Plot A	B	C	H
A	39.9	39.6	40.8	40.1		**		**
B	57.7	49.9	48.4	52.0	**		**	
C	40.8	46.2	38.0	41.7		**		**
D	47.8	45.8	42.9	45.5		*		*
E	46.0	44.0	43.2	44.4		**		**
F	51.6	49.2	41.7	47.5	*		*	
G	51.4	48.8	41.7	47.3	*			*
H	55.3	59.5	46.0	53.6	**		**	

Table 23. Yield of unhusked grains

Plot	Yield of unhusked grains (ton/ha)				Significant difference from		
	Repl. I	II	III	Average	Plot A	C	H
A	1.76	1.78	1.91	1.82			*
B	2.76	2.08	1.95	2.26	*	*	
C	1.83	1.93	1.46	1.74			*
D	2.01	1.91	1.78	1.90			*
E	2.14	2.07	1.38	1.87			*
F	2.41	1.96	2.17	2.18		*	
G	2.46	2.17	1.83	2.15			
H	2.50	2.84	1.96	2.43	*	*	

Plot C, where the insecticide was applied 3 times only in the nursery bed. The tendency was almost the same as in the height and culm length mentioned above. The yield in Plot H was significantly different from that in Plots A, C, D, and E, and that in Plot C was significantly different from Plots B, F, and H. The results are shown in Table 23.

5) Vector population.

In the nursery bed the vector population was surveyed 3 times by sweeping and once by a suction catcher. The vectors, *N. virescens*, were very few on August 13 and 18, and the difference between the treated and untreated plots was not clear. On August 20, 17 adults of *N. viruescens* were found in the untreated plot; only one in the treated plots. According to the results obtained by suction catcher on August 22, 84 adults of *N. virescens* and 1,855 nymphs were found in a 1.5×1.5 m square of the nursery bed where insecticide was not applied, while only one adult and one nymph were found in the treated plots. Most of the leafhopper nymphs mentioned above were of *N. virescens*. The results are shown in Table 24.

In the paddy field the numbers of vectors on 20 hills were counted on September 26. There were very few adults of *N. virescens* and no significant difference was observed among plots. Large numbers of nymphs of *Nephotettix* spp. were found in Plots A and C, where the insecticide was not applied in paddy field; some nymphs were found in Plot D and E, where the insecticide was applied on August 26 and September 2, and on September 2 and 9, respectively. The number of nymphs in Plot A and C was significantly different from that in the other plots. The results are shown in Table 25.

Table 24. Population of leafhoppers in nursery beds

Species of insects	Insecticide application	Number of insects			
		Aug. 13*	18*	20*	22**
Nephotettis virescens	Applied	1	3	1	1
	Non-applied	4	4	17	84
Nephotettix nigropictus	Applied	0	0	0	0
	Non-applied	1	0	3	6
Recilia dorsalis	Applied	0	4	6	13
	Non-applied	2	3	2	55
Other leafhoppers	Applied	1	6	2	24
	Non-applied	5	4	5	78
Leafhopper nymphs***	Applied	0	0	0	1
	Non-applied	0	15	27	1,855

*: Number of insects collected by sweeping 20 times with an insect net.
**: Number of insects collected by suction catcher in 2.25 m².
***: Most were *Nephotettix virescens*.

Table 25. Population of *Nephotettix* spp. in paddy field

Plot		Number of insects on 20 hills				Significant difference from	
		Repl. I	II	III	Total	Plot A	C
Adult	A	1	1	1	3		
	B	0	0	0	0		
	C	1	3	1	5		
	D	0	0	2	2		
	E	0	1	0	1		
	F	0	1	0	1		
	G	0	1	0	1		
	H	0	0	0	0		
Nymph	A	18	54	22	94		
	B	0	0	0	0	*	*
	C	10	21	82	113		
	D	1	0	3	4	*	*
	E	7	5	2	14		*
	F	0	0	0	0	*	*
	G	0	0	0	0	*	*
	H	0	0	0	0	*	*

5. Damage analysis on Yellow Orange Leaf Virus diseased rice plant

In the Central Plain of Thailand, it is said that rice marketing considerably decreased after the severe epidemics of Rice Yellow Orange Leaf Virus (YOLV) disease. The yield of diseased plants in pot tests was reported to be reduced by 70% in Taichung Native 1 and 40% in Leuang Tawng (Wathanakul *et al.* 1970a).

The present chapter deals with damage analysis on YOLV diseased plants in order to clarify the actual condition causing yield decreases.

Materials and Methods

The rice variety Taichung Native 1 was used throughout the experiment. YOLV-diseased and healthy plants, 10 plants each, were collected at harvesting stage from the paddy field which was used for the insecticide application test reported in the previous chapter. Culm length, every internode length, panicle length, and grain weights of all the tillers of these plants were measured and also the numbers of fertile grains on some of the panicles were counted.

In this chapter, the panicle length means the length between the apex and the neck of panicle. The 1st internode, as used here, is the part between the panicle neck and the node from which flag leaf develops. The other internodes are called successively 2nd, 3rd, 4th, and 5th internode. Culm length means the height to the panicle neck.

Experimental Results

1) Internode length and panicle length of YOLV-diseased plants.

The internode lengths and panicle length of diseased and healthy plants are shown in Table 26. The average culm length of diseased plants, which is the sum of the internode lengths, was 38.8 cm, being 15% shorter than that of healthy ones, 49.3 cm. The average lengths of internodes of diseased plants were as follows: the 2nd internode 8.2 cm and 41% shorter than in the healthy plants; the 3rd internode 3.1 cm and 53% shorter; the other

Table 26. Average panicle lengths and internode lengths of YOLV-diseased plants

	Length (cm)			Percentage diminution (%)
	Diseased plants	Healthy plants	Difference	
Number of tillers surveyed	224	195		
Panicle	18.5	20.9	2.4	11.4
1 st internode	19.6	23.7	4.1	17.3
2 nd internode	8.2	13.9	5.7	41.0
3 rd internode	3.1	6.6	3.5	53.0
4 th internode*	1.2	2.0	1.4	45.2
5 th internode*	1.2	2.0	0.8	40.0
Total of internode length	33.8	49.3	15.5	31.4

*: The number of tillers measured was less than that shown in the top part of table.

Table 27. **Average panicle lengths and internode lengths of YOLV-diseased plants for various classes of culm length**

Range of culm length (cm)	Number of tillers surveyed	Panicle length (cm)	Internode length (cm)					
			1st	2nd	3rd	4th	5th	Total
Diseased plants								
40.0—49.9	34	21.4	22.0	11.0	4.1	2.2	1.6	40.9
30.0—39.9	147	18.9	20.4	8.2	3.2	1.7	1.2*	34.7
20.0—29.9	36	15.0	15.8	6.2	2.1	1.5	1.2*	26.8
10.0—19.9	7	13.8	9.0	2.6	1.6	1.2	1.0	15.4
Healthy plants								
60.0—69.9	31	22.8	28.4	18.6	8.7	3.5	2.3	61.5
50.0—59.9	83	21.9	25.2	15.8	7.3	3.2	2.1	53.6
40.0—49.9	45	19.9	22.1	12.3	6.0	3.0	1.7	45.1
30.0—39.9	27	19.0	19.0	8.0	4.4	2.6	1.6*	35.6
20.0—29.9	9	14.9	14.7	4.6	2.4	2.1*	2.0*	25.8

*: The number of tillers measured was less than that shown in the left part of table.

internodes were also shorter in the diseased plants than in the healthy ones. Shortening of the 2nd internode was largest in actual length, while the 3rd internode showed the largest rate of dwarfing. The average panicle length of diseased plants was 2.4 cm, being 11.4% shorter than that of healthy ones.

The measurements of culms are summarized in Table 27. If we compare the diseased and the healthy plants, in the same range of culm lengths, the 1st and 2nd internodes in the diseased plants are mostly a little longer, while the 3rd, 4th and 5th internodes are a little shorter than in the healthy ones. The panicles are almost of the same length or a little longer in the diseased plants.

2) Relationship between culm length and panicle length of YOLV-diseased plants.

Relationships between culm length and panicle length of the diseased and the healthy plants were assessed. The correlation coefficients were $r=0.668^{**}$ and $r=0.575^{**}$, respectively. Seven tillers of the diseased plants shorter than 20 cm in culm length were excluded from the calculation. It is clear that the panicle length in both diseased and healthy plants become longer as the culm length become longer.

Average panicle lengths were calculated for every class of culm length, and are shown in Fig. 7. Broken lines indicate the parts where the averages were obtained from 10 or less plants. Therefore, the averages in these parts were not so accurate. In the diseased plants, the slope of the panicle length to culm length curve is steeper in the range of culm length 26—34 cm than in the range of above 38 cm. In the healthy plants, because of small number of available tillers in this range, the result is not accurate, but the same tendency can be observed as with the diseased plants. In the healthy plants for culm lengths above 42 cm, the curve becomes linear, with a slope smaller than in the range of 34 cm or less culm lengths.

3) Relationship between the culm length and grain weight of YOLV-diseased plants.

As for the relationship between culm length and grain weight in the diseased and the healthy plants, the correlation coefficients were $r=0.724^{**}$ and $r=0.603^{**}$, respectively. Among the tillers of diseased plants, 7 tillers of less than 20 cm in culm length were excluded from the calculation.

The average grain weights in every class of culm length for both the diseased and healthy

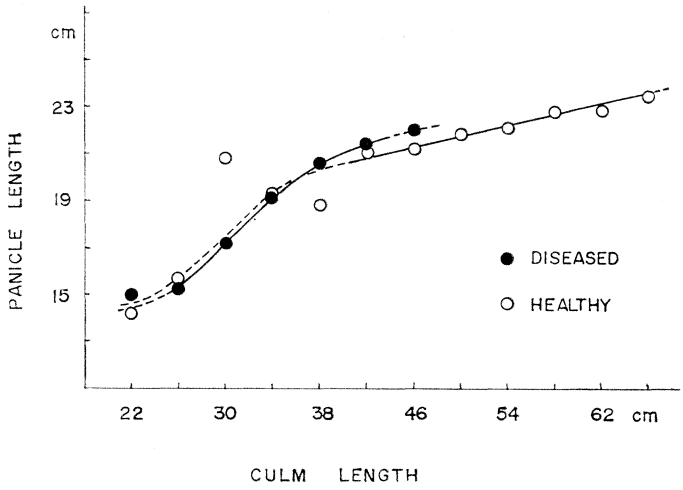

Fig. 7. Comparison of average panicle lengths of YOLV-diseased plants with those of healthy ones at various culm lengths

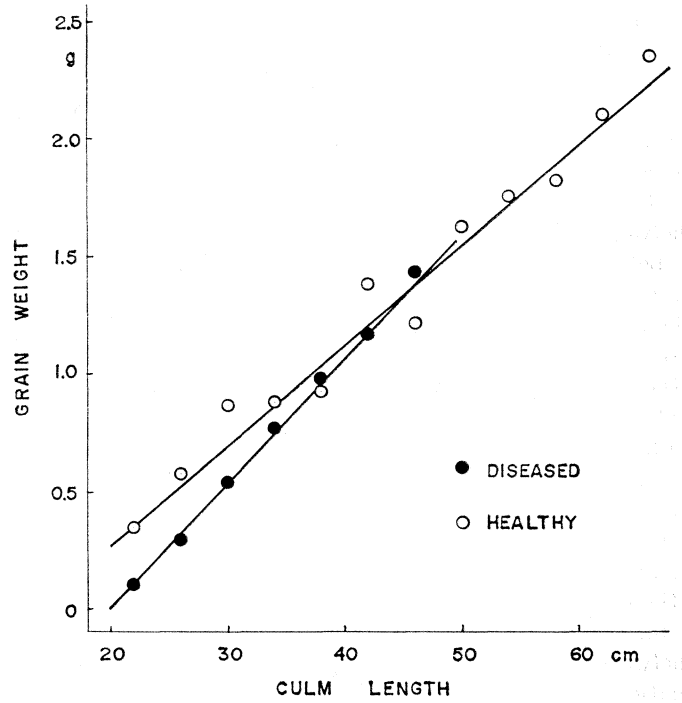

Fig. 8. Comparison of average grain weight per panicles of YOLV-diseased plants with those of healthy ones at various culm lengths

Table 28. Average grain weight of YOLV-diseased plants for various classes of culm length

Class value of culm length (cm)	Diseased plants		Healthy plants	
	Number of tillers surveyed	Grain weight* (g)	Number of tillers surveyed	Grain weight* (g)
66 (64.0—67.9)			4	2.35
62			27	2.10
58			31	1.82
54			38	1.75
50	1	0.9	33	1.62
46	8	1.43	24	1.21
42	25	1.17	16	1.38
38	50	0.98	10	0.92
34	68	0.77	14	0.87
30	42	0.54	5	0.86
26	17	0.29	3	0.57
22	5	0.10	5	0.34
Total and average	216	0.79	210	1.54**

*: Average grain weight on a panicle.
**: Average grain weight in the classes 66—50 cm was 1.82 g; that in the classes 46—22 cm was 1.04 g.

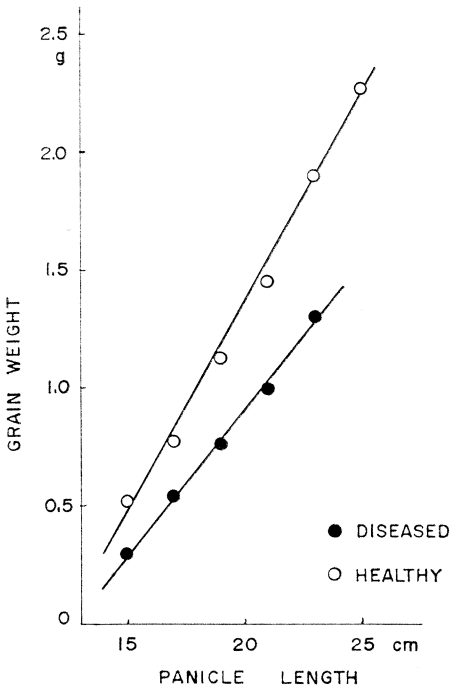

Fig. 9. Comparison of average grain weight per panicle of YOLV-diseased plants with those of healthy ones at various panicle lengths

plants are shown in Fig. 8. It is evident that grain weight is directly proportional to culm length, especially in the diseased plants, and also that grain weight is always lower in diseased plants than in healthy ones when compared at the same culm length.

The overall average grain weight on a panicle, shown in Table 28, was 0.71 g on the diseased plants and 1.54 g on the healthy ones. The diseased plants yielded about 40% less than the healthy ones on a single panicle. In order to compare the grain weight on plants at the same culm length, the average grain weight of the healthy plants of the range of culm length 46—22 cm, the same length range as the diseased ones, was calculated. It was 1.04 g, being still higher than in the diseased plants.

4) Relationship between panicle length and grain weight of YOLV-diseased plants.

Relationship between panicle length and grain weight of the diseased and the healthy plants were also assessed. The correlation coefficients were r=0.643** and r=0.655**, respectively.

The average grain weight for every class of panicle length in the range of 15—25 cm is shown in Fig. 9. Within this range, the grain weight increase is proportinal to the panicle length increase in both diseased and healthy plants. The grain weight of diseased plants is always lower than that of healthy plants in every class of panicle length. In the range of panicle lengths shorter than 15 cm, which is not shown in this figure, comparison could not be made because only few tillers were available.

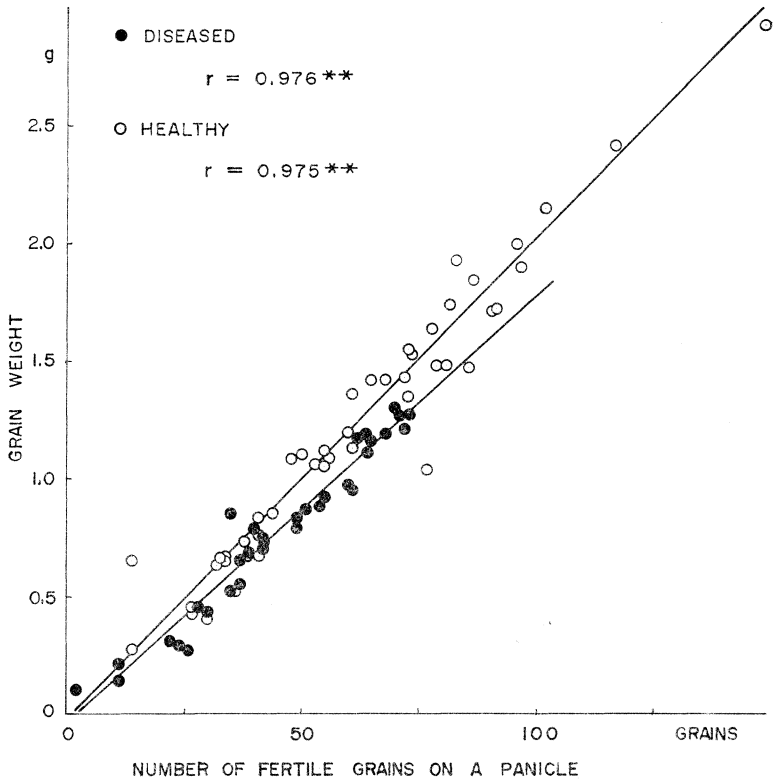

Fig. 10. Correlation between grain weight per panicle and number of fertile grains of YOLV-diseased and healthy plants

5) Relationship between grain weight per panicle and number of fertile grains in YOLV-diseased plants.

The number of fertile grains was counted on 33 panicles from diseased plants and on 45 panicles from healthy ones. Most of the panicles contained both fertile and sterile grains, but only the weight of fertile grains was used, because the weight of sterile grains was negligible.

The relationship between grain weight per panicle and the number of fertile grains is shown in Fig. 10. The correlation coefficients were r=0.976** on the diseased plants and r=0.975** on the healthy ones. In both cases, the grain weight increases proportionally with the number of fertile grains. The rate of increase is smaller in the diseased plants than in the healthy ones.

The weight of 1,000 fertile grains was also calculated from the above data, (this is therefore not the conventional 1,000 grains weight); it was 17.1 g for the diseased plants and 19.8 g for the healthy ones. There were more sterile grains on diseased plants than on healthy ones, although the difference was not calculated.

6) Relationship among average culm length, yield, and disease percentage in the field.

Relationships among average culm length, yield, and disease percentage in the test plots

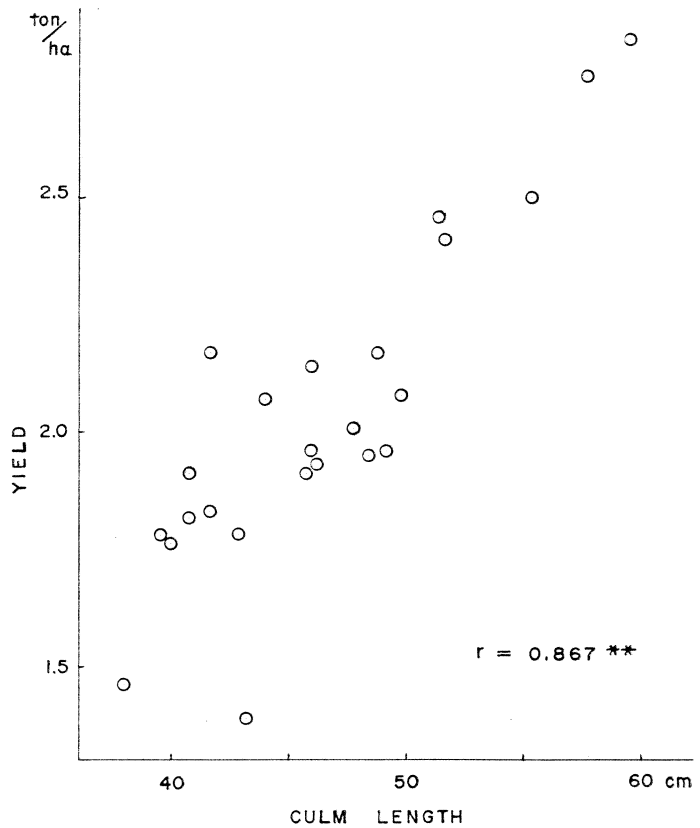

Fig. 11. Correlation between yields and culm lengths in the plots of the insecticide application test discribed in Part A-4

were analyzed, and the results were compared with the results obtained in analyses at tiller level as described above in this chapter.

The correlation coefficient between the average culm length and yield was r=0.867**, as shown in Fig. 11, and proved to be in line with the result obtained in the "Relationship between culm length and grain weight" described above.

The correlation coefficient between the average culm length and the disease percentage was r=−0.851**, as shown in Fig. 12, the culm length decreases proportionally with the increase of disease percentage. The disease percentage used here was the percentage of abnormal hills 61 days after transplanting. The abnormal hills contained, as mentioned in the former chapter, hills which showed clear symptoms and also those which were suspected to be diseased because of vague symptoms. This result shows that the average culm length decreases proportionally with the disease severity. The relationship between the yield and the disease percentage is shown in Fig. 13. The correlation coefficient was r=−0.766**; the yield decreases proportionally with the increase of disease percentage.

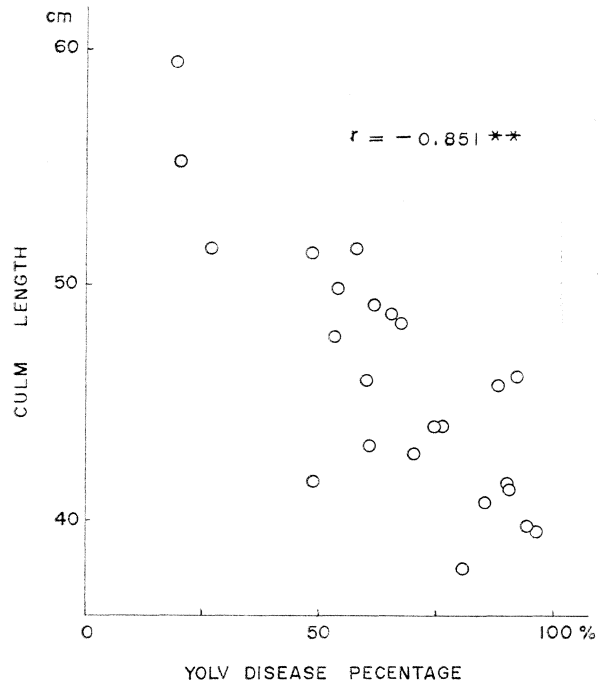

Fig. 12. Correlation between culm lengths and YOLV-disease incidence in the plots of the insecticide application test discribed in Part A-4

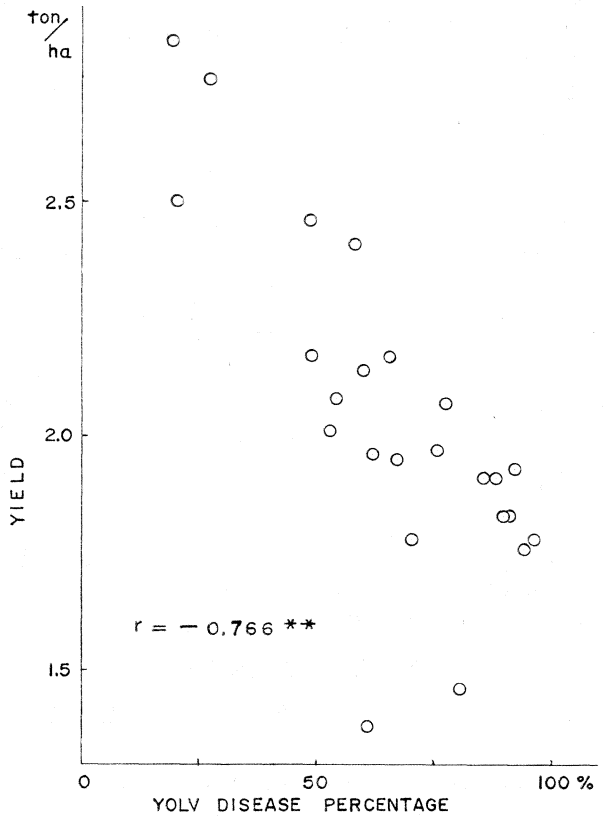

Fig. 13. Correlation between yields and YOLV-disease incidence in the plots of the insecticide application test described in Part A-4

Part B. Laboratory studies on Rice Yellow Orange Leaf Virus disease

6. Transmission of Rice Yellow Orange Leaf Virus by three kinds of vectors

The vectors of Rice Yellow Orange Leaf Virus (YOLV) were reported to be *Nephotettix virescens* (=*N. impicticeps*), *N. nigropictus* (=*N. apicalis*), *Recilia dorsalis* (=*Inazuma dorsalis*), and a mealy bug (an unidentified species of *Pseudococcidae*). *N. virescens* was reported to transmit YOLV with minimum acquisition and inoculation feeding periods of 20 and 15 minutes, respectively, and retain infectivity for 6 days at maximum (Wathanakul *et al.* 1967c, Wathanakul 1969). Thus, YOLV is non-persistent or semi-persistent in the vector, but not mechanically transmissible.

As for similar viruses in the South and Southeast Asia, transmission tests by leafhoppers

were done in Philippines (Rivera *et al.* 1965), in Malaysia (Singh 1969), in Indonesia (Rivera *et al.* 1968), in Bangladesh (Mukhopadhyay *et al.* 1970), and in India (John 1968), and *N. virescens* was found to be the major vector.

We also conducted comparative transmission tests by the above mentioned leafhopper vectors. The results are presented in this chapter.

Materials and Methods

The leafhoppers used were *Nephotettix virescens*, *N. nigropictus*, and *Recilia dorsalis*. These were reared in mass on young rice seedlings which were changed twice a week.

As for the test on YOLV transmission ability, adults or 4th to 5th instar nymphs of these leafhoppers were fed on YOLV-diseased leaves for one day for virus acquisition, and then individually transferred to a healthy rice seedling, Taichung Native 1 at 3-leaf stage, in a test tube, and fed for one day for virus inoculation. The seedlings were then transplanted in pots in a screen-house and kept under observation until symptom appearance.

In the transmission tests on *N. nigropictus* and *R. dorsalis*, successful transmission was judged by the following procedure. As the symptoms caused by transmission using these two species were very mild in most cases, back-inoculation was made from the plants showing the mild symptoms to rice seedlings again by *N. virescens*. Only in cases where the back-inoculated plants showed typical symptoms, positive score was recorded.

These 3 kinds of leafhoppers, especially *Nephotettix* species, were carefully reidentified individually at the time of inoculation feeding.

Experimental Results

As for *N. virescens*, 361 female and 452 male adults were tested, and 53.1% and 50.6%

Table 29. YOLV transmission by *Nephottix virescens*

Test No.	Female adult		Male adult		Nymph	
	Number of insects tested	Number of insects which transmitted YOLV	Number of insects tested	Number of insects which transmitted YOLV	Number of insects tested	Number of insects which transmitted YOLV
I	46	25	47	31		
II	6	3	9	5		
III	21	16	66	42		
IV	30	8	29	9		
V	23	12	60	27		
VI	31	13	26	11		
VII	28	10	3	2		
VIII	46	15	65	20		
IX	91	72	98	56		
X	9	4	35	23	46	30
XI	30	14	14	3	8	8
XII					25	14
XIII					38	30
XIV					43	30
Total	361	192	452	229	160	112
Percentage of transmission		53.1%		50.6%		70.0%

were found to transmit YOLV, respectively; also, 70.0% out of 160 nymphs, as shown in Table 29. In Test IV, mildly stunted young plants with mildly yellowed leaves were used and YOLV was transmitted by 8 out of 30 female and 9 out of 29 male adults. On the other hand, in Test IX clearly yellowed leaves of plants at heading stage were used and the results were 72 out of 91 female and 56 out of 98 male adults. These differences in transmission rates were considered to be caused by difference in virus concentrations in diseased leaves used for the virus acquisition source. This shows that the adult *N. virescens* transmits YOLV very effectively from leaves where the virus concentration is high. The rate of transmission in nymphs, however, did not flactuate as much as in adults, ranging from 14/25 to 8/8.

The efficiency of YOLV transmission was compared between female and male adults, excepting Test II, VII, and X, because of insufficient number of insects tested. The female adults showed higher transmission ability in 5 out of 8 tests, the male adults did in two tests, and almost equal ability in one test. In percentage of transmission calculated from the total number in all the 11 tests, the female adults also showed a higher ability.

N. nigropictus also transmitted YOLV, but the percentage of transmission was very low.

Table 30. YOLV Transmission by *Nephotettix nigropictus*

Test No.	Female adult		Male adult		Nymph	
	Number of insects tested	Number of insects which transmitted YOLV	Number of insects tested	Number of insects which transmitted YOLV	Number of insects tested	Number of insects which transmitted YOLV
I	14	0*(1)**	58	1(1)		
II	28	0	19	0		
III	29	0	68	0	50	0(1)
IV	48	0	55	0	119	1(1)
V	110	0	56	0(1)	5	0
VI			53	1		
Total	229	0(1)	309	2(2)	174	1(2)
Percentage of transmission	0%		0.7%		0.6%	

*: Symptoms appeared and virus was successfully back-inoculated.
**: Symptoms appeared but back-inoculation was unsuccessful.

Table 31. YOLV transmission by *Recilia dorsalis*

Test No.	Number of insects tested	Number of insects which transmitted YOLV
I	60	0
II	93	0
III	117	2
IV	44	0
V	120	3
VI	150	1
VII	143	2
Total	727	8
Percentage of transmission		1.1%

It was 0.4% in 538 adults and 0.6% in 174 nymphs, as shown in Table 30. The symptoms induced by transmission with *N. nigropictus* were very mild, *i.e.*, slight stunting and faint yellowing different from those in the case with *N. virescens*. Therefore, recovery test to healthy seedlings was done by using *N. virescens* from those plants showing mild symptoms. On the test seedlings, severe and typical symptoms appeared in cases of positive transmission. Besides the positive results, shown in Table 30, there were 3 adults and 2 nymphs which seemed to have transmitted, but the back-inoculations to rice by *N. virescens* were unsuccessful. Every individual of *N. nigropictus* tested was carefully identified, in order to exclude any contamination with *N. virescens*.

The percentage of YOLV transmission in *R. dorsalis* adults was also low and was 1.1%, as shown in Table 31. The symptoms induced by transmission with *R. dorsalis* were also mild, the same as in the case of *N. nigropictus*. The positive results mentioned above were confirmed by recovery tests using *N. virescens*.

7. Effect of temperature on growth period of *Nephotettix virescens* nymphs

The nymph period of *Nephotettix virescens* has been reported to vary according to temperature; it showed optimum growth at 28°C in Japan (Nasu 1964). However, such results obtained in the temperature zone may not be applied to the tropical zone, because of possible presence of biotypes in different climatic zones.

As we planned to improve the techniques of rearing and using nymphs in each instar for transmission tests, it became neccessary to determine accurately instar and nymph periods at different temperatures. It was also necessary for the analysis of seasonal changes in viruliferous vector population described in Chapter 2 of the present report.

The present chapter deals with nymphal growth periods of *Nephotettix virescens* at different temperatures. The experiment was conducted with particular care and technique in handling the insects without injury.

Materials and Methods

Eggs of *Nephotettix virescens* laid in rice leaf sheaths were collected from rearing cages, and were kept in moistened petri dishes until hatching. The hatched nymphs were transferred individually to rice seedlings, Taichung Native 1, one nymph to a seedling in a test tube. The nymphs with seedlings in test tubes were kept in growth cabinets at various constant temperatures of 23, 25, 28, 30, 33, and 38°C, and observed daily.

In order to transfer the hatched nymphs, a small lump of loose cotton fibres attached to the tip of forceps was used. A hatched nymph was induced to creep up the cotton lump and was then transferred with the cotton lump to a rice seedling. The seedling was renewed every 3 or 4 days. The plant part on which the nymph perched was cut off and moved onto a new seedling in a new test tube. An old nymph of about 5th instar was let to move by itself from the dark oil seedling to a new one through the joined openings of the test tubes held in the direction of light. Glass tube suckers conventionally used for transferring small insects were not used in this experiment.

Experimental Results

The number of nymphs escaped, died, or grown to adults are shown in Table 32. In the 1st and 2nd instar, the nymphs were very small and easily lost. Dead bodies in these instars

Table 32. Growth and mortality of *Nephotettix virescens* at different temperatures

Instar	Number of insects observed					
	23*	25**	28*	30**	33*	38°C**
Number of insects died						
1st instar	1	8	6	7	25	55
2nd	0	0	2	1	2	23
3rd	1	0	0	2	5	5
4th	0	0	1	1	9	
5th	0	1	8	0	9	
Total	2	9	17	11	50	83
Number of insects escaped						
1st instar	2	26	3	27	6	0
2nd	0	15	1	9	0	2
3rd	0	5	0	11	3	0
4th	0	6	2	7	2	
5th	0	11	4	5	3	
Total	2	63	10	59	14	2
Number of insects grown to adult						
	6	31	49	49	54	0

*: Experiment I. **: Experiment II.

Table 33. Mortality of *Nephotettix virescens* in each instar at different temperatures

Instar	Mortality (%)					
	23	25	28	30	33	38°C
1st instar	13	20	9	12	24	66
2nd	0	0	3	2	2	28
3rd	13	0	0	3	5	6
4th	0	0	2	2	9	
5th	0	3	12	0	9	
Total	25	23	26	18	48	100

Remarks: The mortality was calculated from the number of nymphs died and those grown to adult stage in Table 32.

were sometimes unable to be found, being hidden in plant parts. The mortality was calculated from the number of nymphs which was observed to be surely dead and that which grew to adults, as shown in Table 33. The escaped or missed nymphs were not included in the calculation. At 38°C no nymph could grow to adult, and at 33°C 48% of nymphs died. At 30—23°C mortality was about equal and ranged from 18 to 26%. In most cases, at every temperature, mortality was high in the 1st instar.

The periods of nymphal instars at different temperatures are shown in Fig. 14. The instar period of male nymphs was in most cases shorter than in female at every temperature.

The overall nymph period of males was shorter than that of females in all cases. The

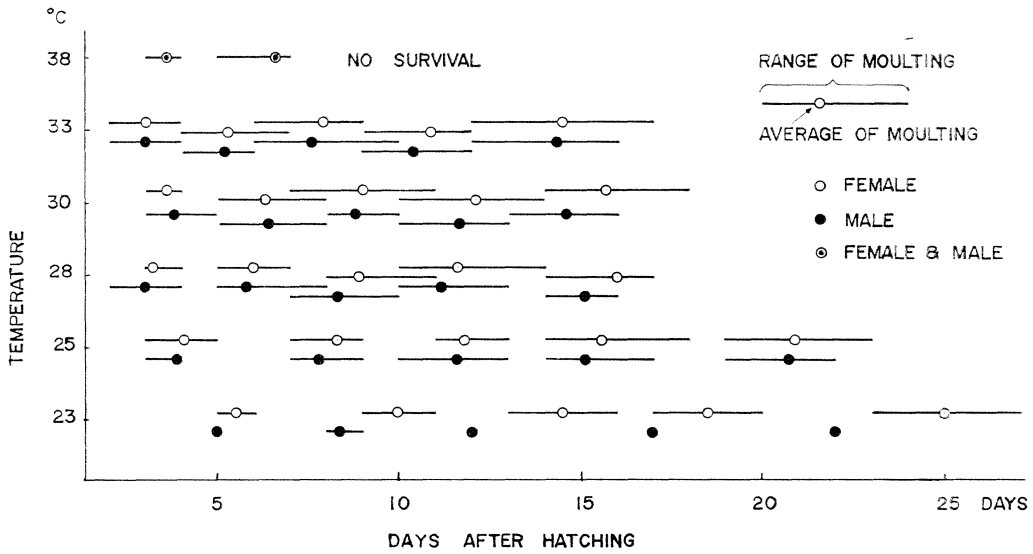

Fig. 14. Duration of each instar period of *Nephotettix virescens* at different temperatures

shortest nymph period was recorded at 33°C, while the nymph periods were longer at lower temperatures. At 33, 30, and 28°C the nymph period was about equal, the difference being only 1.5 days in female and 0.8 days in male. Although nymphs could not grow to adult at 38°C as mentioned above, the periods of 1st and 2nd instars at this temperature were longer than at 33°C.

8. Effect of moulting of *Nephotettix virescens* nymphs on Yellow Orange Leaf Virus transmission

In *Nephotettix virescens*, the nymph was reported to transmit Rice Yellow Orange Leaf Virus (YOLV) more effectively than the adult (Wathanaku *et al.* 1967c). In Rice Tungro Virus in the Philippines, a virus similar to YOLV and transmitted by the same vector as YOLV, viruliferous nymphs are reported to become free from Rice Tungro Virus when the nymphs moult (Ling 1966).

In order to see whether infectivity is lost on moulting also in the case of YOLV, virus retension at the time of moulting in nymphs of each instar was studied.

Materials and Methods

The rice variety Taichung Native 1 and the vector *Nephotettix virescens* were used. The vectors were fed at 25°C because of longer instar period but sufficient growth for the experiment at this temperature as shown in the previous chapter. Younger instar nymphs were handled in the same way as in the previous chapter. Older instar nymphs, in this experiment, were carefully handled by a conventional glass tube insect sucker.

The 1st instar nymphs were transferred to YOLV-diseased leaves immediately after hatching and fed there for 1 day. The 2nd and 3rd instar nymphs were reared on healthy rice seedlings from hatching unitl they reached the intended instar and then fed on diseased

leaves for 1 day. The 4th and 5th instar nymphs, some of the 3rd instar nymphs, and adults were fed on diseased leaves for 1 day, being moved directly from cages for mass rearing.

The vector having fed on diseased leaves was put on a healthy rice seedling, one vector on one seedling in a test tube. The seedling was renewed every day and transplanted in pots in screen-house. From the symptom appearance, virus retention by the vector was judged.

In cases of nymphs gathered directly from mass rearing cages, moulting until adult was observed in order to confirm the nymphal instar at the time of the experiment.

Experimental Results

In the 1st instar, 7 out of 19 nymphs transmitted the virus, and the period of virus retention was one day. In the 2nd instar, 14 out of 22 nymphs transmitted the virus, and the period of virus retention was 2 days in 6 cases and 3 days in 2 cases. In the latter 2 cases no transmission occurred for the first 2 days. In the 3rd instar, 28 out of 36 nymphs transmitted the virus, and the period of virus retention was 3 days in 3 cases and 2 days in 5 cases. The results are shown in Fig. 15.

The 4th instar nymphs, as shown in Fig. 16, transmitted the virus in 34 out of 44 cases and retained the virus for 4 days in one case, for 3 days in 3 cases, and for 2 days in 7 cases. The 5th instar nymph transmitted the virus in 55 out 80 cases, and retained the virus for 5 days in 4 cases, for 4 days in 2 cases, and for 3 days in 19 cases, as shown in Fig. 17.

All virus transmissions ended before or on the moulting day except in 2 cases, one in the 2nd instar and the other in the 3rd instar, as shown in Fig. 15.

The adults retained the virus for 5 days in 3 cases, as shown in Fig. 18, and the percentage of transmission on the 1st day and 2nd day was 37% and 31%, respectively. The percentage decreased on the 3rd day to 13% (Table 34).

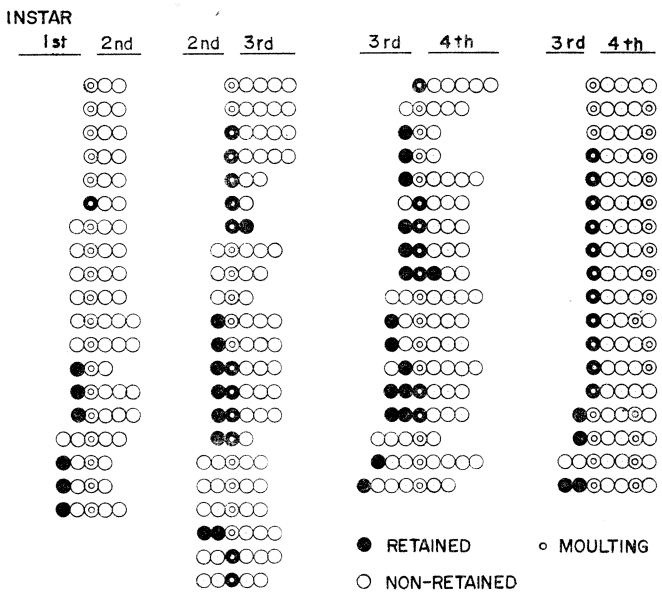

Fig. 15. YOLV retention by *Nephotettix virescens* nymphs in the 1st, 2nd and 3rd instars
(The test at left and two in the middle were done in Traial I, and that at right was done in Trial II.)

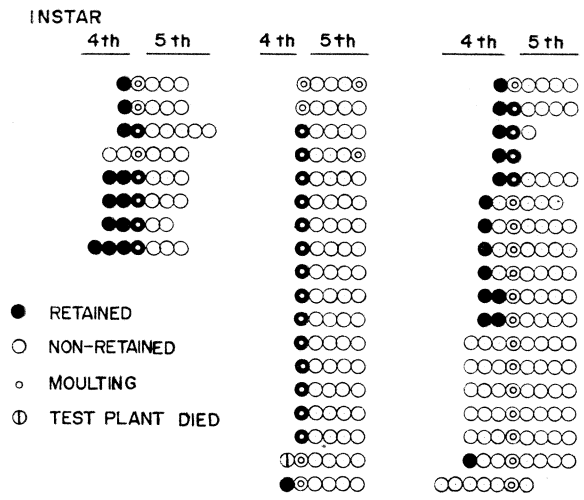

Fig. 16. YOLV retention by *Nephotettix virescens* nymphs in the 4th instar
(The test at left was done in Trial I, and those in the middle and at right were done in Trial II.)

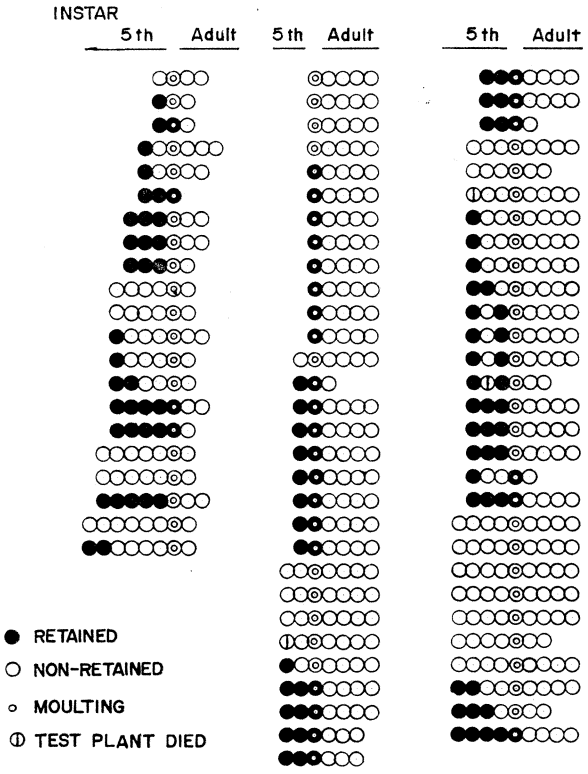

Fig. 17. YOLV retention by *Nephotettix virescens* nymphs in the 5th instar
(The test at left was done in Trial I, and those in the middle and at right were done in Trial II.)

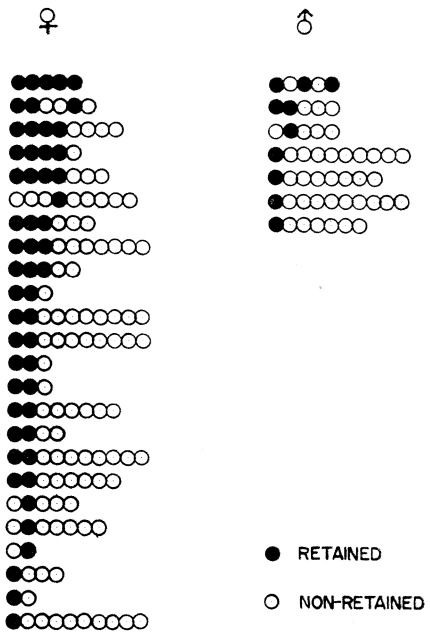

Fig. 18. YOLV retention by *Nephotettix virescens* adults

Table 34. Transmission and retention of YOLV by adults of *Nephotettix virescens*

Days after virus acquisition	Number of vectors tested		Percentage of virus transmission (%)		
	Female	Male	Female	Male	Total
1 day	53	17	38	35	37
2	53	17	38	12	31
3	48	16	15	6	13
4	41	14	12	0	9
5	35	14	6	7	6
6	28	11	0	0	0
7	23	10	0	0	0
8	20	7	0	0	0
9	14	4	0	0	0
10	11	4	0	0	0

Nymphs of all instars transmitted YOLV. The percentage of viruliferous nymphs in the 1st, 2nd, 3rd, 4th, and 5th instar was about 40, 60, 80, 80, and 70%, respectively. The percentage of viruliferous adults was about 40%. As these vectors acquired the virus from different virus sources at different times, these figures can not be compared with each other. However, virus acquisition and transmission ability seemed to be lower in the 1st and 2nd instar nymphs and also in adults than in the 3rd, 4th and 5th instar nymphs.

Virus retention period appeared to become longer as the instars progressed, the longest retention in the 1st, 2nd, 3rd, 4th and 5th instar was recorded to be 1, 3, 3, 4, and 5 days, respectively.

In many cases, virus retention ended on the day of moulting, especially in cases where the nymphs had acquired the virus one or two days before moulting. This suggests that the nymphs lost the virus at the time of moulting, just as in the case of Rice Tungro Virus in the Philippines (Ling 1966). Exceptions were observed with 2 nymphs during the experiment. These 2 nymphs in the 2nd and 3rd instar transmitted the virus until the day after moulting. This may have been due to wrong diagnoses caused by ambiguous symptoms (Chapter 11), or virus re-acquisition after moulting because of the exchange of seedlings at one day intervals (Chapter 9). These 2 nymphs may as well have lost the virus at the moulting as in other nymphs.

In order to assess average period of virus retention by nymphs, the effect of moulting should be excluded. Among 56 nymphs in the 5th instar which could be tested for 3 days before the moulting (including the moulting day), 35 nymphs retained the virus. Among these, 4 retained the virus for 5 days, 2 for 4 days, 19 for 3 days, 6 for 2 days, and 4 for one day. In the adults, among 31 individuals which acquired the virus, 3 retained the virus for 5 days, 4 for 4 days, 3 for 3 days, 14 for 2 days, and 7 for one day. From the comparison of virus retention between the nymph and the adult, the average period of virus retention by 5th instar nymphs was longer than that in the adults, when moulting did not occur during the period.

From the results obtained, nymphs are considered to be as important as adults in the spread of YOLV disease in nursery beds and in paddy fields.

9. Latent period of Rice Yellow Orange Leaf Virus in plant

There are two distinct phases in virus disease development: incubation period and latent period. The incubation period is the time needed for symptoms to develop, that is, the time from infection to symptom appearance. The latent period is the time needed for the infected plant to become infectious, that is, the time from infection to becoming infectious (Plank 1963). In this chapter, the results of our experiment to determine the latent period in Rice Yellow Orange Leaf Virus (YOLV) disease are described.

Materials and Methods

1) Ten-day-old seedlings of Taichung Native 1 and adults of the vector *Nephotettix virescens* were used throughout the experiment. The method was as follows:

2) Preparation of latent-period-test-plant.

Healthy vectors were fed on YOLV-diseased leaves for 3 days (from Friday afternoon to Monday morning). Five vectors, thus made to be viruliferous, were transferred to a healthy seedling and fed for 3 hours (from 8:30—9:00 to 11:30—12:00 on Monday). The seedling thus inoculated was then tested for consecutive 3 days to determine latent period, and was called latent-period-test-plant in this experiment.

3) Determination of latent period.

a) Tests just after inoculation.

Five healthy vectors were fed on the latent-period-test-plant for 3 hours (from 12:00—12:30 to 15:00—15:30 on Monday). These vectors were transferred to a healthy test seedling and fed on it until the next morning (Tuesday morning). The test seedling was transplanted in a pot in a screen-house and virus acquisition and transmission was judged from symptom

appearance on this seedling.

b) **Tests 1, 2, and 3 days after inoculation.**

The latent-period-test-plant, used for the test just after inoculation mentioned above, was used repeatedly. By the same method and time-schedule, the vectors were fed on it for 3 hours and tested for virus acquisition and transmission. (The tests 1, 2, and 3 days after inoculation were started on Tuesday, Wednesday, and Thursday, respectively.)

4) **Checking of the latent-period-test-plant.**

The latent-period-test-plant, used for the tests mentioned above from Monday to Thursday, was transplanted in a pot in a screen-house on Thursday afternoon, and checked for virus infection by observing symptom appearance.

Experimental Results

YOLV was isolated from 44 out of 50 inoculated latent-period-test-plants 3 days after inoculation as shown in Table 35. Among the 6 plants from which virus was not isolated, one plant (Plant No. 4) proved to be infected; virus might have been isolated if the test was continued for one day or more. Three plants (Plant No. 18, 31 and 35) among the 6 were not infected, while the remaining 2 plants (Plant No. 1 and 3) could not be designated because of death of the plants. If we assume that these 2 plants were infected, the plants successfully infected were 47 in total. (Among the plants from which virus could be isolated, one plant (Plant No. 41) showed no symptoms, but this plant was considered to be infected.)

From 36 out of 47 infected plants, virus was isolated 2 days after inoculation, 10 after

Table 35. Acquisition of YOLV from inoculated plants by *Nephotettix virescens*

Infection checking of inoculated plants*	Virus acquisition: Days after inoculation 0	1	2	3 days	Number of cases observed (Subtotal)	Remarks
+	+	+	+	+	1 (1)	Plant No. 11
+	−	+	+	+	7	
?	−	+	+	+	2 (9)	
+	−	−	+	+	19	
−	−	−	+	+	1	Plant No. 41
+	−	−	+	?	3	
?	−	−	+	?	3 (26)	
+	−	−	−	+	6	
?	−	−	?	+	1	
+	−	?	−	+	1 (8)	
+	−	−	−	−	1	Plant No. 4
?	−	−	?	−	1	Plant No. 1
?	−	?	−	−	1 (3)	Plant No. 3
−	−	−	−	−	1	Plant No. 18
−	−	−	−	?	2 (3)	Plant No. 31, 35
				Total	50	

+ : Infection of test seedling. − : No infection. ? : Test seedling died.
* : These are called latent-period-test-plants in the text.

one day, and one (Plant No. 11) just following or within 7 hours after inoculation.

These results suggest that many of the inoculated plants became infectious 2 days after inoculation, and almost all the plants after 3 days. Symptom of YOLV appears after an incubation period of 7 to 10 days on young seedlings. Exceptionally, very faint symptom may appear after 4 days. The latent period of YOLV was found to be 2 days in most cases, being distinctly shorter than the incubation period.

The shortest latent period was 7 hours in a single case out of 47 in this experiment, but there was obtained several minutes' latent period in another series of tests made by Wathanakul (unpublished). However, such cases are rather exceptional, and it is considered that usually 2 days after inoculation the inoculated virus or multiplied virus are translocated to other plant parts, and becomes available to the vectors.

10. Host range of Rice Yellow Orange Leaf Virus

In Thailand, rice plants are mostly cultivated in rainy season, and only in a limited area can be cultivated also in dry season. In the latter instance where rice plants are cultivated twice a year, Rice Yellow Orange Leaf Virus (YOLV) can be transmitted serially from rice to rice. In other cases where rice is cultivated only in rainy season, YOLV must overseason by some other way. Since the vector insect cannot be the overseasoning host because of non-persistency (*cf.* Chapter 8), the virus should overseason on some plant hosts.

The present chapter deals with the result of our experiments on host range of YOLV.

Materials and Methods

Host plants were obtained as follows: 1) Plants were grown from seeds in a screen-house, 2) young seedlings were collected from fields, or 3) with some perennial grasses, small propagative shoots were collected from fields. In the latter two cases, 2) and 3), the plants were grown to maturity in order to confirm the species identification after heading. The height of the plants was 4—12 cm at the time of virus inoculation.

The vectors used were adults and 5th instar nymphs of *Nephotettix virescens*, obtained by mass rearing and tested to be non-viruliferous. For back-inoculation tests from inoculated plants, the rice variety Taichung Native 1 was used at 2—3 leaf stage, with a height of 10—15 cm.

For inoculation, the vectors were first fed on diseased rice leaves for one day and then transferred to the plant to be inoculated, 20 vectors on each plant in a test tube, and fed for one day. After inoculation, the plants were transplanted in pots and kept in a screen-house or outdoors for observation.

The back-inoculation tests were made when symptoms clearly appeared on inoculated plants, or after one month in cases of plants without symptoms. Leaves of the inoculated plants were individually put in a test tube, then 20 or 30 vectors were introduced in the test tube and fed for one day. The vectors were then transferred to healthy rice seedlings, 3—5 vectors per seedling, and fed for one day. The rice seedlings were transplanted in pots in a screen-house, and kept under observation for symptom appearance.

In the case of wild rice, one vector was fed on a plant for one day both in the inoculation- and back-inoculation tests, different from the tests with other plants.

Experimental Results

Twenty one species of Gramineae and 2 species of Cyperaceae were tested, and the result

Table 36. YOLV inoculation tests on various plants

Family, species and variety	Replication	Number of plants tested	Symptoms induced	Result of back-inoculation** Replication 1	2	3	4
GRAMINEAE							
Brachiaria mutica		6	—	—	—		
Chloris barbarta	I	5	—	—			
	II	4	—	—			
Cynodon dactylon		5	—	—			
Dactyloctenium aegyptium	I	1	—	—			
	II	4	—	—			
Digitaria adscendens	I	5	—	—	—		
	II	4	—	?			
Echinochloa colonum	I	3	STT, VCL, ROL	?	?	?	?
	II	6	STT, VCL, ROL	?			
	III	5	—	—			
	IV	6	STT, VCL, ROL	?	?		
	V	4	STT, VCL, ROL	?			
Echinochloa crus-galli	I	3	STT, VCL, ROL	?			
	II	1	STT, VCL, ROL	?			
Eleusine indica	I	5	—	—			
	II	3	—	—			
Eragrostis tenella	I	5	—	—			
	II	3	—	—			
Eriochloa annulata	I	5	—	—			
	II	5	—	—			
Hordeum sativum							
var. Musashinomugi	I	4	—	—			
	II	3	—	—			
var. Sekitori Sai 1	I	2	—	—			
	II	1	—	—			
Imperata cylindrica	I	3	—	—			
	II	4	—	—			
Leersia hexandra		5	ivc	—	—	—	
Leptochloa chinensis	I	7	ivc	?	?		
	II	1	—	—			
Leptochloa panicea	I	5	ivc	—			
	II	6	VBD	?			
*Oryza rufipogon****			STT, IVC, YEL	+			
Panicum repens		5	—	—			
Paspalum distichum	I	5	—	—			
	II	5	—	?			
Setaria glauca		5	—	—			
Triticum vulgare							
var. Komugi Norin 61		5	—	—			
var. Ushiokomugi	I	6	—	—			
	II	1	—	—			
Zoisia japonica		4	—	?			
CYPERACEAE							
Cyperus difformis		5	—	—			
Fimbristylis miliacea		5	—	—			

* IVC: Interveinal chlorosis, ROL: Inward rolling of leaves, STT: Stunting, VBD: Veinbanding, VCL: Veinclearing, YEL: Yellowing, —: No symptom. Capitals and small letters indicate severe and mild symptoms, respectively.

** +: Virus was successfully back-inoculated. —: Virus could not be back-inoculated. ?: Symptom on back-inoculated rice was dubious.

*** For detail see Table 37.

is shown in Table 36. Only wild rice, *Oryza rufipogon*, showed clear symptoms and the virus was successfully back-inoculated from this plant to rice. Indecisive results were obtained in some other plants.

In *Echinochloa colonum*, a weed which grows in paddy fields in shallow water and on dikes, slight mosaic was observed on newly developed leaf about 10 days after inoculation. After one month, the plant showed stunting, with short leaf blades and vein-clearing on the leaves. Some leaves showed inward rolling. This species was tested 5 times and the same symptoms were observed in 4 tests. The symptoms are shown in Fig. 19. Back-inoculation tests to rice, Taichung Native 1, were repeatedly made, but typical and clear symptoms were not obtained. The symptoms obtained as shown in Fig. 20, A and B, were much milder, and the plants became almost normal-looking within several days. Furthermore, virus could not be transmitted further from these plants to rice. Control plants were inoculated at the same time by the same method but diseased rice plant was used as the source. These control plants showed typical symptoms, as shown in Fig. 20, C and D, and did not became normal-looking for 2—3 weeks.

In *Echinochloa crus-galli*, a weed which grows in canals and paddy fields in deep water, slight dwarfing began one week after inoculation, and later veinclearing appeared, as shown in Fig. 21. The symptoms were milder than in *E. colonum*. Virus failed to be back-inoculated, although sometimes the inoculated rice seedlings showed dubious symptoms as in the case of *E. colonum*.

In *Leersia hexandra*, a weed which grows along canals and dikes, very slight interveinal chlorosis was observed, but the virus could not be back-inoculated.

In *Leptochloa chinensis*, a weed in paddy fields and on dikes, the inoculated plants showed a very slight dwarfing just after inoculation, but later became nomal-looking. Newly de-

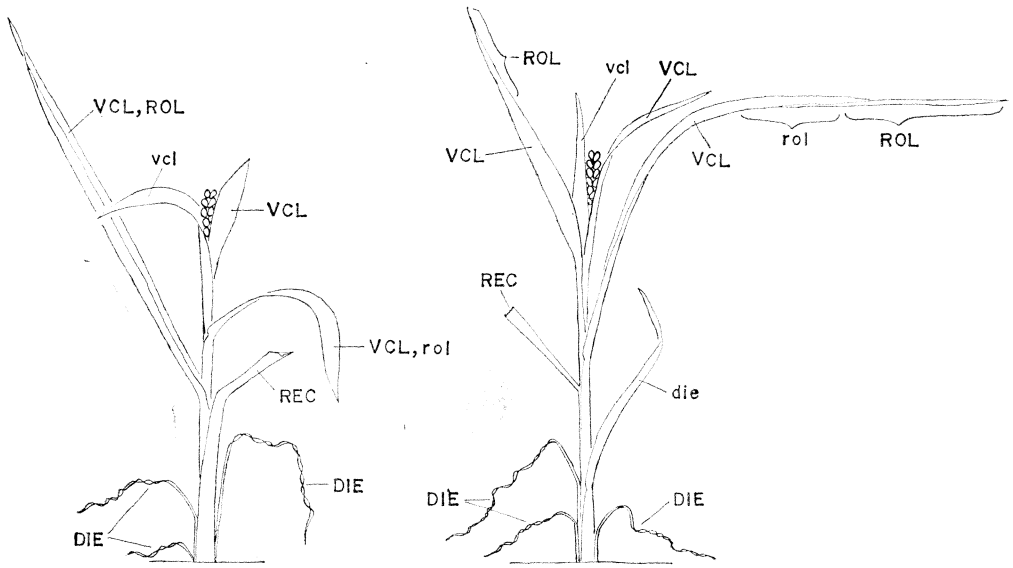

Fig. 19. Symptoms on *Echinochloa colonum* caused by inoculation with YOLV

DIE: Dead leaf, REC: Leaf used for back-inoculation, ROL: Inwards rolling, VCL:Veinclearing. Capitals and small letters indicate severe and mild symptoms respectively.

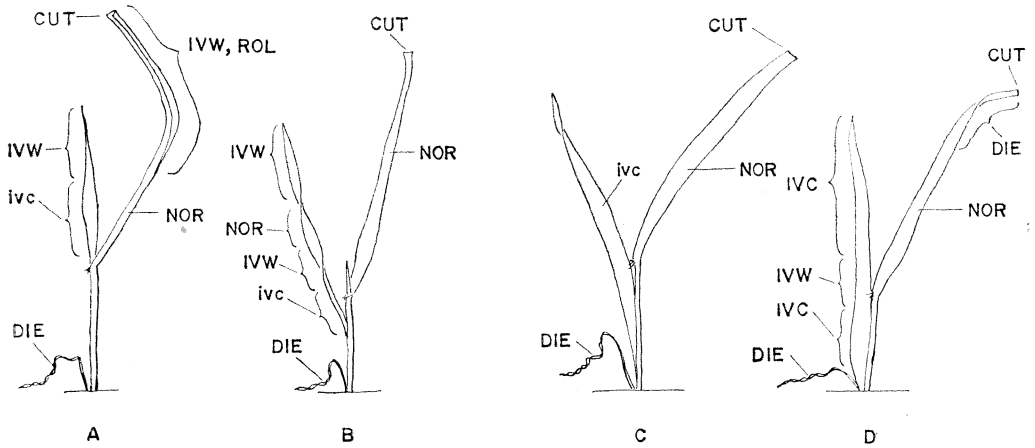

Fig. 20. Symptoms on Taichung Native 1 caused back-inoculation from *Echinochloa colonum* A and B, or by inoculation with YOLV

A and B: Back-inoculated from *E. colonum*, C and D: Inoculated with YOLV
CUT: Cut before inoculation, DIE: Dead leaf, IVC: Interveinal chlorosis, IVW: Interveinal white specks, NOR: Normal, ROL: Inwards rolling
Capitals and small letters indicate severe and mild symptoms, respectively.

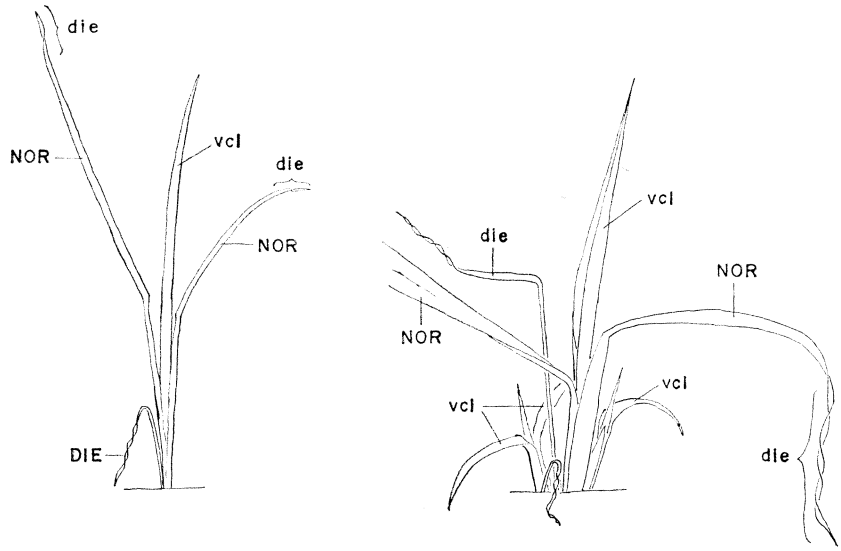

Fig. 21. Symptoms of *Echinochloa crus-galli* caused by inoculation with YOLV

DIE: Dead leaf, NOR: Normal, VCL: Veinclearing. Capitals and small letters indicate severe and mild symptoms, respectively.

veloped leaves showed veinclearing. Virus could not be back-inoculated, although the inoculated rice seedlings showed dubious symptoms as in the case of *E. colonum*.

In *Leptochloa panicea*, a weed on dikes, slight interveinal chlorosis and veinbanding were observed on newly developed leaves, but the virus could not be back-inoculated, except

Table 37. YOLV inoculation tests on wild rice, *Oryza rufipogon*

Collection No.	Locality	Date of inoculation (1971)	Number of plants tested	Number of plants diseased	Result of back-inoculation
1	Chonburi	July 15	3	1	
1	Chonburi	July 27	6	3	
2	Maklang	July 15	3	2	
2	Maklang	July 27	13	7	
3	Nakhon Sawan	July 26	13	11	+
3	Nakhon Sawan	July 27	4	4	
4	Pitsanuloke	July 27	6	6	
5	Ratubri	July 5	10	3	+
5	Ratburi	July 26	3	3	
5	Ratburi	Aug. 5	7	4	+
6	Sankhaburi	July 27	5	2	
6	Sankhaburi	Aug. 5	4	3	
7	Saraburi	July 5	10	3	+

Remarks: All the collections showed clear symptoms, stunting, white specks, interveinal chlorosis, and yellowing.
The localities are shown in Figure 22.

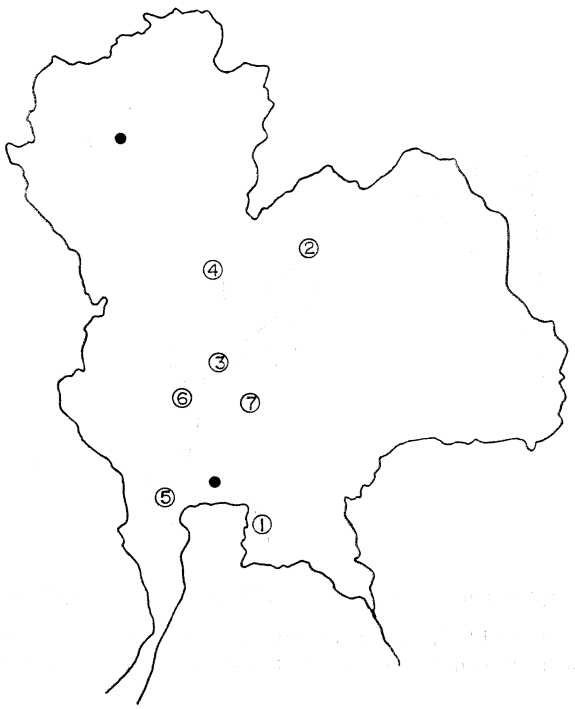

Fig. 22. Localities of wild rice collections
1: Chonburi, 2: Maklang, 3: Nakhon Sawan, 4: Pitsanuloke, 5: Ratburi, 6: Sankhaburi, 7: Saraburi. Solid cirlces show the locations of Bangkok and Chiang Mai.

one suspicious case.

Wild rice, *Oryza rufipogon* (=*O. perennis*, *O. fatua*, *O. sativa* form. *spontanea*), grows in canals, moats, and ponds of shallow and deep water close to paddy fields. The results of inoculation tests are shown in Table 37. The plants were collected from seven localities of Thailand as shown in Fig. 22. All the collections inoculated showed typical symptoms as described below, and virus was successfully back-inoculated from 3 collections tested.

Symptoms on wild rice were almost the same in all the collections. The inoculated plant was at first dwarfed, and newly developed leaves showed interveinal white specking and fading. These then changed to interveinal chlorosis, and later general yellowing. The symptoms are shown in Fig. 23, and are almost the same as those on cultivated rice, except for the severe white specks.

Digitaria adscendens, *Zoisia japonica*, and *Paspalum distichum* showed no symptom, but sometimes dubious symptoms were observed in back-inoculated Taichung Native 1. The first is a weed on dikes, the second is a grass used for lawns, and the third is a weed in shallow water and along dikes.

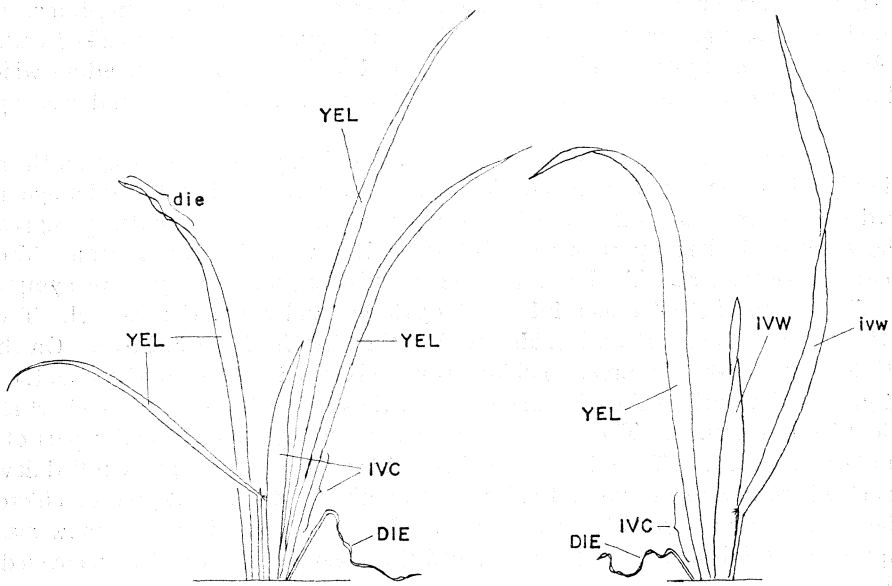

Fig. 23. Symtoms on *Oryza rufipogon*, wild rice, caused by inoculation with YOLV

DIE: Dead leaf, IVC: Interveinal chlorosis, IVW: Interveinal white specks, YEL: Yellowing. Capitals and small letters indicate severe and mild symptoms, respectively.

11. Symptoms of Yellow Orange Leaf Virus disease on young rice seedlings in screen house

The symptoms of Rice Yellow Orange Leaf Virus (YOLV) disease have already been described in detail (Lamey 1967, Wathanakul *et al.* 1967c), which are in general almost the same as in Tungro in the Philippines, Penyakit Merah in Malaysia, and Mentek in Indonesia.

These symptoms are also similar to some physiological disorders, and it is usually necessary for exact YOLV disease diagnosis to test for the transmissibility as in certain other virus diseases. The initial symptoms of YOLV disease on seedlings in screen house are sometimes not clear, and the symptoms later diminish in many cases.

The present chapter deals with YOLV symptoms observed incidentally in the course of experiments.

1) Symptoms on inoculated seedlings of Taichung Native 1.

Various symptoms caused by YOLV on Taichung Native 1 are shown in Fig. 24. These are the most typical symptoms of YOLV on young seedlings under experimental conditions. The virus was inoculated to the seedlings of 2.7—3.0 leaf stage, which means the 3rd leaf has developed by 70—100% (The 1st leaf means the primary leaf without blade). The 3rd leaf rarely showed very slight interveinal chlorosis, in most cases remaining normal. The sheath of the 4th leaf, which developed just after inoculation, was distinctly stunted, sometimes the leaf blade emerging directly from the soil level. The balde of the 4th leaf showed interveinal chlorotic dottings, interveinal chlorosis, and sometimes gradually turned yellowish orange.

Masking of symptoms is shown in Fig. 25. After showing typical symptoms, the plant begins to show masking. In the case shown here, the symptoms were masked earlier than usual. At the beginning of masking, newly emerged leaf showed vein banding which later changed to dark green. The sheath of the next leaf was of normal length, and was apparently healthy.

The usual symptom progress is shown in Fig. 26, although in this case again the masking occurred only a little earlier than usual. The virus was inoculated at 3.0 leaf stage, top part of the 3rd leaf having been cut away before inoculation. Chlorotic dottings appeared between the veins at the basal part of the 4th leaf blade 7 days after inoculation. The sheath of this leaf was very short. Twelve days after inoculation, more conspicuous symptoms appeared. The sheath of the 4th leaf did not elongate yet and was under the soil. The sheath of the 5th leaf was also short and hidden within the sheath of the 3rd leaf. On the blade of the 5th leaf, mosaic-like interveinal chlorosis was observed. After 15 days, as the 3rd leaf its vivid green color and its sheath came off from those of other leaves, the short sheath of the 5th leaf became visible. The 6th leaf came out normally from the collar part of the 5th leaf, as in healthy plants. The edges of the 4th and 5th leaf blades slightly rolled downwards at the part where severe interveinal chlorosis was observed. The degree of chlorosis was about the same as that 3 days before. After 21 days, the plant began to show masking of the symptoms: the 6th leaf looked normal, and the sheath of the 5th leaf recovered a little in length.

2) Symptoms on inoculated seedlings of Japanese rice varieties.

YOLV was inoculated to 9 Japanese rice varieties, Hatsunishiki, Honenwase, Kanto No. 51, Koshihikari, Manryo, Norin No. 25, Norin No. 29, Sasashigure, and Tangin. The leaf stages of seedlings at inoculation were 4.0 on Norin No. 29, 3.2—3.4 on Honenwase and Manryo, and 3.0 on the other varieties. Plant heights at the time of inoculation were 17—20 cm in Honenwase, Norin No. 29, and Sasashigure, 8 cm in Tangin, and 14—15 cm in the others. The check variety, Taichung Native 1, was 15 cm high and at 3.0 leaf stage.

All the Japanese varieties except Kanto No. 51 are *japonica*. Kanto No. 51 is a hybrid between *japonica* and Chinese *indica* varieties, while Taichung Native 1 is a Chinese *indica* variety.

Symptoms appeared 7 days after inoculation. The sheath of the newly developed 4th leaf was remarkably short, and there appeared on the blade interveinal faint chlorotic dottings. After 2 weeks the symptoms became clearer. When compared with Taichung Native 1, the

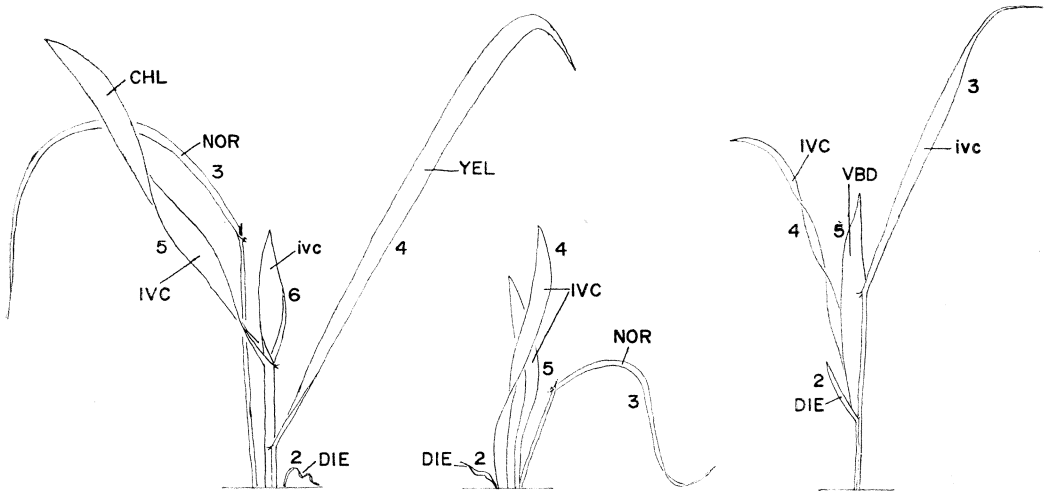

Fig. 24. Typical symptom of YOLV on Taichung Native 1

CHL: Chlorosis, DIE: Dead leaf, IVC: Interveinal chlorosis, NOR: Normal, VBD: Veinbanding, YEL: Yellowing, 2 to 6: Leaf number.
Capital and small letters indicate severe and mild symptoms, respectively.

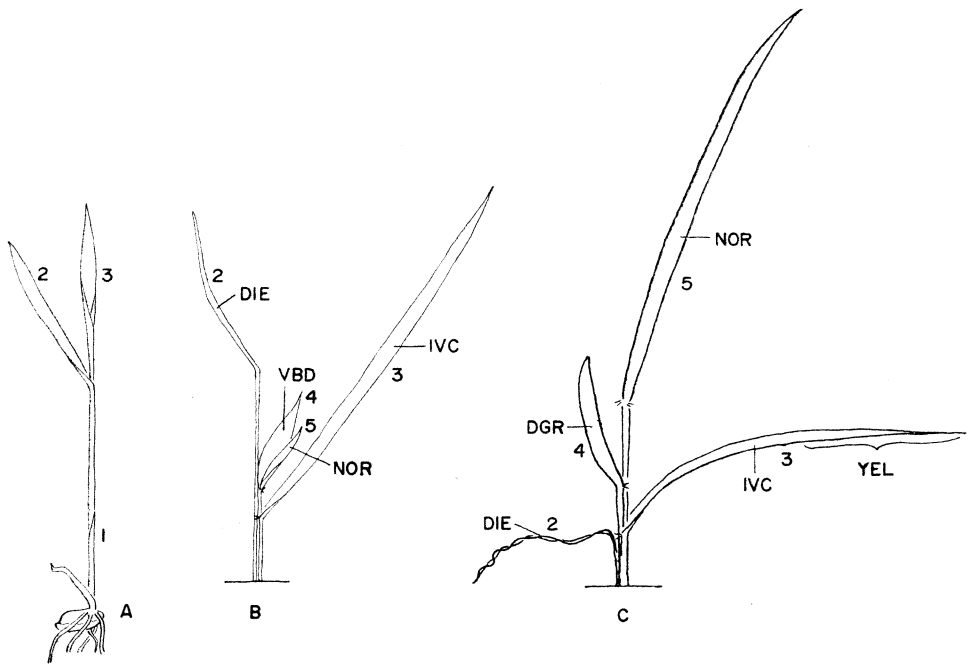

Fig. 25. Early masking of YOLV symptoms on Taichung Native 1

DGR: Dark green, DIE: Dead leaf, IVC: Interveinal chlorosis, NOR: Normal, VBD: Veinbanding, YEL: Yellowing, 1 to 5: Leaf number.
Capital and small letters indicate severe and mild symptoms, respectively.
A: Just after inoculation, B: After 12 days, C: After 15 days.

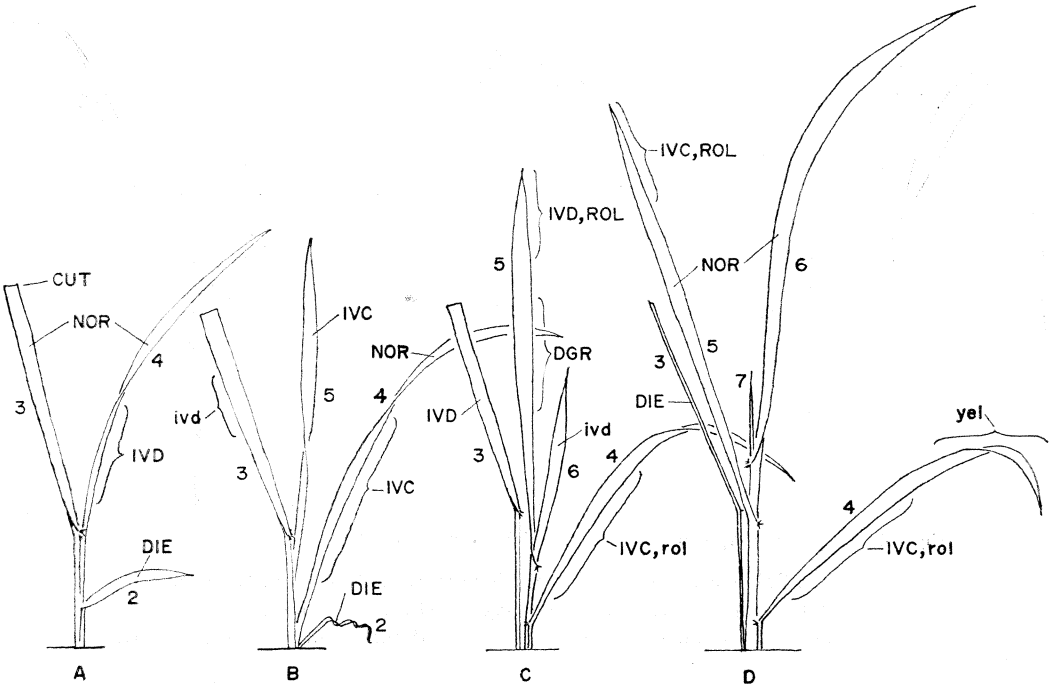

Fig. 26. Usual masking of YOLV symptoms on Taichung Native 1

CUT: Cut before inoculation, DGR: Dark green, DIE: Dead leaf, IVC: Interveinal chlorosis, IVD: Interveinal chlorotic dottings, NOR: Normal, ROL: Downward rolling, YEL: Yellowing, 2 to 7: Leaf number. Capital and small letters indicate severe and mild symptoms, respectively. A: Seven days after inoculation, B: After 12 days, C: Agter 15 days, D: After 21 days.

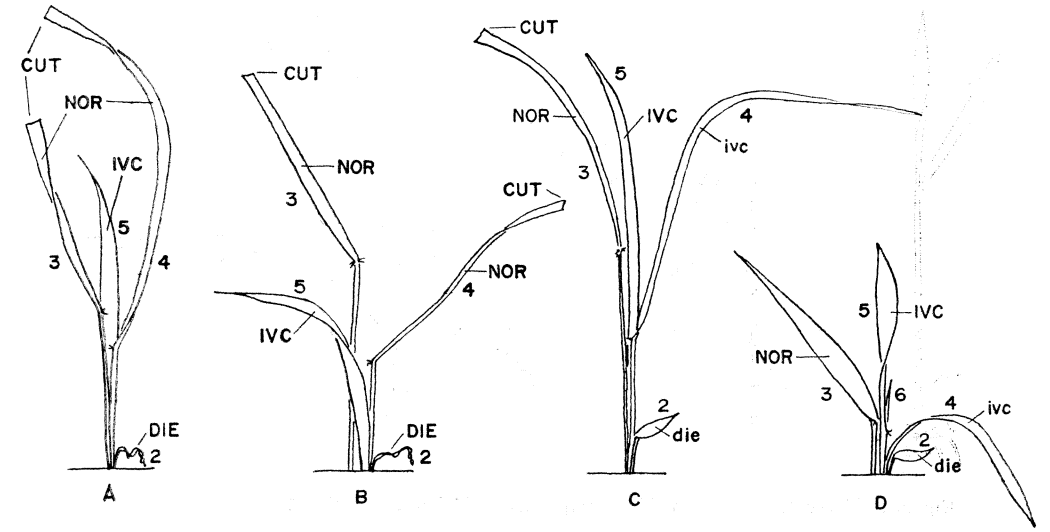

Fig. 27. The symptoms of YOLV on Japanese rice varieties

CUT: Cut before inoculation, IVC: Interveinal chlorosis, NOR: Normal, 2 to 6: Leaf number. Capital and small letters indicate severe and mild symptoms, respectively. A: Honenwase, B: Norin No. 29, C: Manryo, D: Tangin.

degree of sheath shortening in the Japanese varieties was about the same, and that of interveinal chlorosis was also about the same or a little lighter except Norin No. 29. No remarkable differences in symptom expression were observed among the 9 varieties. The symptoms on some varieties are shown in Fig. 27.

3) Symptoms on inoculated seedlings of Thai rice varieties.

The varieties used were Gow Ruang 88, Leuang Tawng, Muey Nawng 62 M, Gam Pai 15, Nahng Prayah 70, and Khao Pahk Maw 148. These are native varieties recommended by the former Rice Department. At the time of inoculation, the leaf stages of seedlings were 3.2 on Muey Nawng 62 M and 3.0 on the other varieties. Plant heights were 28 cm in Gam Pai 15, 21 cm in Gow Ruang 88 and Nahng Prayah 148, and 25 cm in the other ones. As these varieties were inoculated at the same time as the Japanese varieties, and Taichung Native 1 described in the former paragraph, the results can be compared.

Symptoms appeared 7 days after inoculation. The sheath of the 4th leaf was shortened, and interveinal chlorotic dottings appeared on the blade as in the Japanese varieties. The chlorotic dottings were clearer than those on the Japanese varieties. After 2 weeks the interveinal chlorotic dottings changed into interveinal chlorosis, again as in the Japanese varieties. The symptoms on some varieties are shown in Fig. 28.

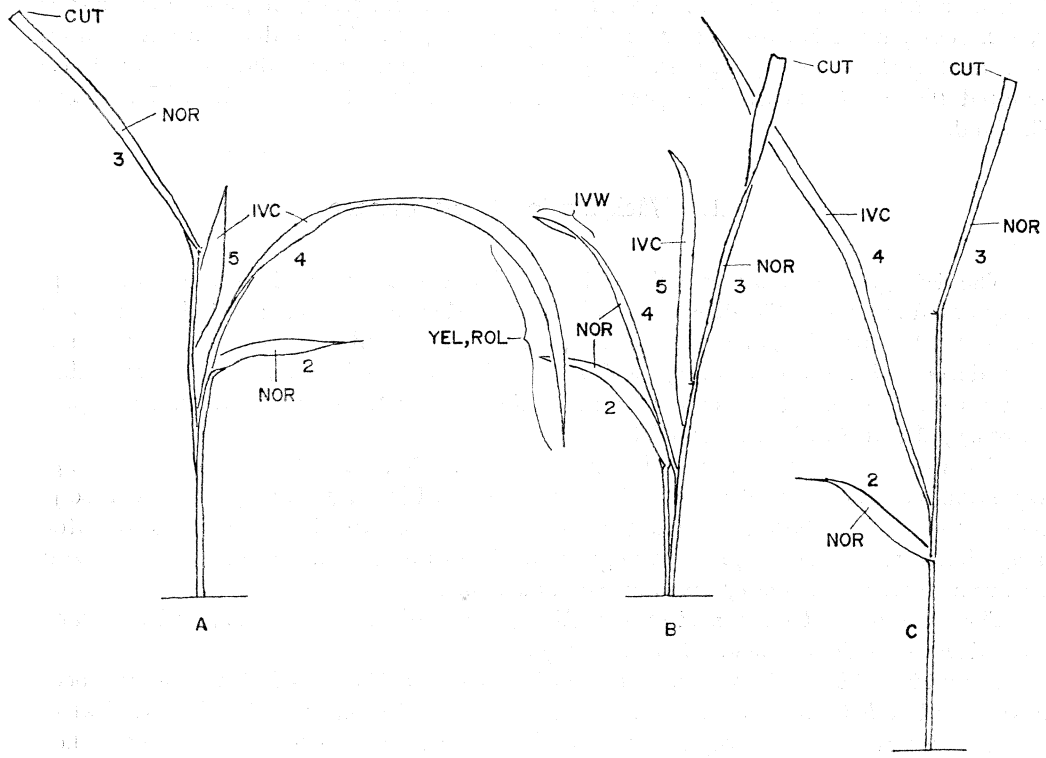

Fig. 28. Symptoms of YOLV on Thai rice varieties

CUT: Cut before inoculation, DIE: Dead leaf, IVC: Interveinal chlorosis, IVW: Interveinal white specks, NOR: Normal, ROL: Downward rolling, YEL: Yellowing, 2 to 5: Leaf number.
Capital and small letters indicate severe and mild symptoms, respectively.
A: Khao Pahk Maw 148, B: Leuang Tawng, C: Gam Pai 15.

V. Discussion and conclusion

In Thailand, 4 kinds of rice virus diseases have been identified on the basis of insect transmission and absence of visible pathogens under light microscope (Wathanakul *et al.* 1967b, 1971). We tried to determine the nature of pathogens of these 4 diseases, namely Yellow Orange Leaf Virus (YOLV) disease, Orange Leaf disease, Yellow Dwarf disease and Grassy Stunt disease. YOLV disease was proved, by cooperation with Dr. Yasuo Saito to be caused by a virus of nearly spherical shape, 30 milimicrons in diameter (Saito 1970, Saito *et al.* 1971, Nabheerong *et al.* 1971). In the tissues of Yellow Dwarf diseased plants, virus particles could not be found, but mycoplasma-like structures were observed. In the tissues of Orange Leaf diseased plants examined, neither virus particles nor mycoplasma-like structures could be found, and the pathogen has not yet been determined. The 4th disease, Grassy Stunt, has not been observed during the 3 years of the present work, so that no experiments on pathogen determination could be done. YOLV disease is therefore the only virus disease of rice in Thailand which has been confirmed from aetiological standpoint.

Diseases similar to YOLV disease are widely distributed all over South and Southeast Asia, in Bangladesh, India, Indonesia, Malaysia, the Philippines, *etc.*, and sometimes called by different names, Mentek, Penyakit Merah, Tungro, *etc.* These diseases have tentatively been put together in a single group, Tungro group, but further classification has not been attempted (Ou *et al.* 1969). The present study is concerned only with YOLV disease of Thailand.

1. Vicissitude of the disease

The data given in Chapter 1 show that YOLV disease occurred most severely on plants transplanted in June. The disease was also observed on plants transplanted in July, August, and September, but the percentage of diseased hills decreased each month. On plants transplanted in October and December, the disease seldom occurred and symptoms were indistinct. No disease was observed on plants transplanted in May or November of 1969, nor in January, February, March, April and May of 1970.

The disease occurrences mentioned above coincided with the population changes of *Nephotettix virescens*, except for the plants transplanted in May. Symptoms on these plants were not distinct, however, probably because they were already at the stage of internode elongation at the time of *Nephotettix* migration. The disease occurrence was not in accordance with prevalences of *N. nigropictus* nor *Recilia dorsalis*.

The varieties and lines used except RD-2, were resistant to YOLV, and the plants recovered from the disease before booting stage.

In paddy fields in the Central Plain of Thailand, in 1969, YOLV disease first occurred in July and ended with the start of the dry season. Farmers mostly planted Thai native rice varieties, which are susceptible to the disease and hence never recover even at heading stage. The results given here are considered to be in line with this field vicissitude of YOLV disease in the Central Plain in 1969, except for symptom recovery due to varietal resistance.

Since YOLV disease was discovered in 1964 at Bangkhen, Bangkok, its distribution in Thailand extended year after year, 1965, 1966, and 1967 (King 1966, Lamey 1967). In 1968, the disease occurred very severely, and in 1969 it was also severe. In 1970, the disease again occurred severely, but a little milder than in 1969. It was not observed in April and

May, and only rarely in June even on susceptible varieties, but became prevalent in July, August, September, and October. The severest infection was observed on plants transplanted in August, the peak of disease occurrence being later than in 1969. The disease diminished in the dry season.

In 1971, the disease was not prevalent and was only rarely observed in April, May, June, July, and August. Symptoms were observed on susceptible varieties in September, October, November, and December in experimental fields, with the peak occurrence in October. In farmers' paddy fields, the disease was observed rarely only in October.

These changes in disease incidence from year to year may also be influenced by changes in the prevalence of the vectors, which in turn depend on climatic conditions. The severity of YOLV disease occurrence was reported to be related to the numbers of vectors collected by light traps in June and July (Yoshimeki 1971). If data on YOLV disease and vector occurrences could be accumulated for some years, relationships between disease occurrence and climatic conditions would probably become clear.

2. Vicissitude of viruliferous vectors

Four species of *Nephotettix* have been reported from Thailand, *N. virescens*, *N. nigropictus*, *N. parvus*, and *N. malayanus* (Kawase 1971). The former 2 species inhabit paddy fields, while the latter 2 species live in weed communities. Only one species of the genus *Recilia*, *R. dorsalis*, has been reported to be distributed in paddy fields in Thailand.

Three different kinds of leafhopper vectors have been reported, *N. virescens*, *N. nigropictus*, and *R. dorsalis* (Wathanakul *et al.* 1967c, Wathanakul 1969). However, the last two species are less common in nursery beds and paddy fields, and show a rather weaker virus transmissibility (Chapter 6). Vicissitudes of these two species are also not in accordance with disease prevalences in paddy fields (Chapter 1). Therefore, only *N. virescens* was tested in our survey of viruliferous vector population.

N. virescens is generally only scarecely found in the dry season. However, when nursery beds were cultivated serially throughout year and adjacent to each other, the leafhoppers were observed in nursery beds even in the dry season, though the population was low at that time, (Table 4).

In Bangkok, the rainy season usually begins in June and continues until October. In the dry season, there are three distinct climatic phases: cool season in December, intermediate season from January to February, and hot season from March to May. Although these seasonal sequences fluctuate according to year, during the present experiment it was almost normal, as mentioned above.

In the first half of the rainy season from June to August, average air temperature (AV Temp.) declined to 28—29°C, the vector population was high and percentage of virutiferous vectors (VV%) gradually increased. In the second half of the rainy season from September to November, AV Temp. was 26—29°C, and the vector population remained high, VV% was also as high as 40% or more. In December, cool season, AV Temp. suddenly dropped to 23—26°C, and at the same time VV% suddenly decreased. In January and February, AV Temp. increased to 26—29°C, and VV% gradually increased to attain 40%. In the hot season from March to May, AV Temp. reached as high as 29—31°C, and the vector population and VV% also became low.

These relationships between VV% and AV Temp. may be interpreted as follows: 1. AV Temp. of 26—29°C is optimum for both the increase in VV% and for population build-up. 2. The VV% decrease in the cool season may be ascribed to decreased activities of the vectors,

since there are still abundant infection sources, but inactive vectors should have less chances of virus acquisition and infection. 3. The VV% decrease in the hot season is considered to be due to the decreased vector population as a result of lowered fecundity and enhanced mortality at high temperatures. The low vector population should be conductive to fewer chances of virus acquisition and infection.

These results are in line with the results of experiments described in Chapter 7. The optimum temperature for the growth of *N. virescens* was found to be 28—30°C. When the temperature declined to 25—28°C, the nymphal period became longer. When the temperature increased to 30—33°C, nymph mortality became high. At 38°C, no nymphs survived.

3. Time of infection in nursery beds and paddy fields

The disease usually appears at the beginning of the rainy season. In 1970, the disease incidence in June seemed to be less than in the previous years, but the disease continued until September. The experiments described in Chapter 3 was made under these conditions. Seeds were sown on June 26 in Experiment I, and on August 20 in Experiment II. The rice variety Leuang Tawng, susceptible variety used for these experiments, is non-photosensitive, so that the growth period is almost unchanged irrespective of cultivating season.

The plants in plots covered with screen cages (screen-cage plots) were apt to show symptoms faster than those in open plots. Temperatures in the screen-cage plots were lower than in the open plots, although the difference was less than 1°C. Slight shading from the sunshine in the scree-cage plots seemed to protect the transplanted seedlings from wilting and accelerate rooting activity. The period from disease infection to symptom appearance, namely the incubation period, is considered to be much more influenced by the shading than by the temperature difference. The percentages of diseased hills in the screen-cage plots and in the open plots were about equal at final stage.

The diseased hills in the screen-cage plots included two kinds of plants; those with symptoms which had already appeared in the nursery bed and those with symptoms which appeared after transplanting. Since both were infected in the nursery, the maximum percentage of diseased hills in the screen-cage plots was the percentage of infected hills at the time of transplanting.

The percentage of diseased hills in the nursery bed was not so high; 0.01% and 0.08% in Exp. I and II, respectively, after 25 days from sowing and 0.07% and 3% in Exp. I and II, respectively, after 36—38 days. On the other hand, the percentage of infected hills at the time of transplanting, was 0% and 24.8% in Exp. I and II, respectively, after 25 days from sowing, and 5.9% and 93.2% in Exp. I and II, respectively, after 35—36 days. Thus a big difference was observed between the percentage of diseased hills in the nursery bed and the percentage of infected hills at the time of transplanting. The difference can not be explained by the length of incubation period. It seems that in the nursery bed some diseased plants might carry very vague symptoms and are in distinguishable from healthy plants.

In the nursery bed, the vector population was about equal in Exp. I and II, but the percentages of viruliferous vectors in Exp. II were higher than in Exp. I. This explains why the percentage of infected hills in the nursery bed of Exp. II was higher than that of Exp. I.

The percentage of diseased hills in the open plot in the paddy field, in every case, increased to a much higher level than the percentage of infected hills at the time of transplanting. Especially, in case of the plants transplanted 25 days after sowing in Exp. I, infection occurred only in the paddy field and 40.5% of the hills were diseased 37 days after transplanting. This shows that viruliferous vectors migrated into the paddy field and spread the disease.

Vectors in paddy fields adjacent to the experimental fields were proved to be viruliferous, 46% in the adults, showing that vectors in the experimental fields might also have been viruliferous. However, the vector population in the paddy field was very low, as shown in Table 9. It is interesting that such a very low population of vectors resulted in such heavy infections. Although behaviors of the vectors in the tropical zone have not been studied, the vectors in this zone might be more active than those in the temperate zone, at least under certain conditions Besides the vector activity, some characters of the virus, such as the short latent period, (Chapter 9) might also be conductive to acceralated disease spread.

Analysis of the infection rate (Plank 1963) showed that the rate in the nursery bed was 1.9 to 3.8 times higher than that in the paddy field. There must be more chances of infection in nursery beds, as vector populations are usually much higher than in paddy fields. Younger plants also may have higher susceptibility, and moreover, young seedlings show shorter latent perid, 2 days in most cases, as mentioned in Chapter 9. Nymphs of every instar transmit the virus, the 3rd to 5th instar nymphs being most effective, even more effective than adults. (Chapter 6 and 8). All these factors may cause higher infection rate in the nursery bed than in the paddy field.

4. Chemical control of the disease

In the nursery bed where insecticide was not applied, the vectors increased in number and the population reached about 1,800 in 1.5×1.5 m square 22 days after sowing, whereas only 2 vectors were found in the nursery bed where insecticide was applied 3 times, as described in Chapter 4.

At the time of transplanting, 23 days after sowing, no diseased plants were observed in either the treated or non-treated nursery beds. There are possibilities that some infected hills did not show symptoms until after transplanting. The following 4 plots were chosen for the determination of the effect of insecticide application for disease control in the nursery bed. In Plot A, insecticide was not applied in both nursery bed and paddy field. In Plot B, it was not applied in nursery bed but applied 4 times in paddy field. In Plot C, it was applied 3 times in nursery bed but not applied in field. In Plot H, it was applied 3 and 4 times in nuersery bed and paddy field, respectively.

At the first survey on the percentage of diseased hills on September 12 (20 days after transplantation), the percentage in Plot A was higher than in Plot C, and that in Plot B was higher than in Plot H. Although the differences were not significant, this suggested the possibility of higher incidence of infection in the untreated nursery beds. In this experiment, however, the insecticide application in nursery beds was found to be effective in decreasing the vector population, but the effect on the disease control could not be confirmed.

At a later stage, the percentage of diseased hills in Plot A was significantly higher than in Plot B, and that in Plot C was higher than in Plot H. This shows a distinct effectiveness of 4 times isecticide application in paddy fields for disease control.

In the timing and frequency of insecticide application, no significant differences were observed. However, the rate of increase of diseased hills appeared to vary with the timing and frequency of application. The percentage diseased hills in Plots B and H, where the insecticide was applied 4 times, did not increase until several days after the last application, while the percentage in Plots A and C, non-application in paddy field, increased remarkably. In the twice-application plots, plots D and E, the percentage increased very little at first and only gradually later. The percentage in Plots F and G increased about the same as in Plots A and C, non-application plots, and only slightly later. In these 4 plots, Plots D, E,

F, and G, the insecticide was applied respectively, 3 days and 10 days after transplantation, after 10 and 17 days, after 17 and 24 days, and after 3 and 17 days. If the application after 3 days is assumed to be ineffective, the increasing tendency in each plot is in accord with the application timing.

The percentage of "abnormal hills" in Plots A and C, non-application in paddy field, increased remarkably with the lapse of time, being significantly different from the other plots. The percentage in Plots B and H with 4 applications increased gradually, while the percentage in the twice-application plots showed an intermediate increase. No significant difference was observed between the plots with 4 and 2 applications. The tendency is almost the same as in the percentage of diseased hills, despite different standards for symptom rating.

The plant height, culm length, and yield in Plots B and H, 4 applications in paddy field, were the highest and significantly different from Plots A and C, non-application plots. The corresponding data for the twice-application plots showed intermediate values. The yield in non-application plots was about 25% lower than that in plots with 4 applications. Relationships among the yield, culm length, and disease incidence will be discussed in detail in the next article, "Damage analysis of the disease".

Adult vectors were found in all plots except Plots B and H, but in small numbers, corresponding to the result described in Chapter 3. Many nymphs were found in Plots A and C, non-application plots, while some nymphs were also found in Plots D and E. The abundance of nymphs in Plots A and C is quite plausible, but the reason for the presence of nymphs in Plots D and E is rather difficult to understand.

From these results, Sevin Granule applications proved to be effective in decreasing vector population and controlling Rice Yellow Orange Leaf Virus disease in paddy fields. As judged from disease occurrences in nursery beds, the insecticide should be applied there also, in accordance with conventional schedules. The results of this experiment on the timing of isecticide application in the paddy field did not lead to a definite conclusion. However, to start insecticide application 3 days after transplanting seems to be too early, and more effective schedule would be to start a little later.

5. Damage analysis of the disease

In a study of damages caused by Rice Yellow Orange Leaf Virus (YOLV), yield decrease in pot tests was reported to be 70% with Taichung Native 1 and 40% with Leuang Tawng, but factors relating to the decreased yield were not analyzed (Wathanakul *et al.* 1970a). In the present experiment on YOLV disease control by insecticide application, the highest yield was 2.43 tons/ha in the plot where Sevin granules were applied 7 times, while the lowest yield was 1.74 tons/ha in the plots where no insecticide was used, as mentioned in Chapter 4.

In order to analyze factors causing the yield decrease, culm lengths, lengths of all internodes panicle lengths, and grain weights were measured on each tiller of 10 diseased and 10 healthy hills. On some panicles among these, the numbers of fertile grains were also counted.

The average culm length of diseased plants was 33.8 cm, or 31.5% less than in healthy ones. The largest decrease in internode length was observed on the 2nd internode in the actual length and on the 3rd internode in the rate of decrease. However, when both classes of plants were compared at the same culm length, the 1st and 2nd internodes of diseased plants were a little longer, while the 3rd, 4th, and 5th internodes were a little shorter than in healthy ones.

The average panicle length of diseased plants was 18.5 cm, or 11.4% less than in healthy

ones. The panicle length increased in proportion to culm length increase. When compared with each other at the same culm length, the panicle lengths of both classes of plants were almost equal.

The grain weight on a panicle of diseased plants averaged 0.79 g, or about 40% decrease in comparison with healthy ones. If the number of panicles was the same on both diseased and healthy plants, this means that the decrease in yield of diseased plants would be about 40%. Even when yield was compared at the same culm length level and tillers of healthy plants which had longer culms than diseased ones were excluded, the yield was roughly estimated to be about 20% less than in healthy ones.

Correlation coefficient between culm length and grain weight was $r=0.724^{**}$ for the diseased plants and $r=0.603^{**}$ for the healthy plants, respectively. The garin weight in both classes of plants was directly proportional to the culm length. The regression in the diseased plants was 0.0542 and was larger than in the healthy ones which was 0.0426. Both regression lines crossed at the culm length of 46 cm in this experiment. As only a few diseased plants were found to be longer than 46 cm in culm length, it may be that the grain weight on a panicle of diseased plants was always lighter than that of healthy ones at the same culm length, and that the difference became larger as the culm length became shorter.

Correlation coefficients between grain weight and panicle length were $r=0.643^{**}$ and $r=0.655^{**}$ in diseased and healthy plants, respectively. The relationships were linear in the range of panicle length of longer than 15 cm. The rate of increase was smaller in diseased plants and the grain weight of diseased plants was always less than those of healthy ones at the same panicle length in this range.

Correlation coefficients between the grain weight and number of fertile grains on a panicle were $r=0.976^{**}$ and $r=0.975^{**}$ in diseased and healthy plants, respectively. The rate of increase in the grain weight of diseased plants in proportion to the increase in number of fertile grains on a panicle was a little larger than in healthy ones. The weight of 1,000 fertile grains of diseased plants was calculated from these data to be about 13% less than that of healthy ones (This is not the conventional 1,000 grains weight).

From these results it is clear that the yield in YOLV-diseased plants decreases in proportion to shortening of culm length, and that the yield is lower even when compared with healthy plants at the same culm length. Yield decrease is considered to be caused by the decrease in the weight of individual grains as well as by the decrease in number of fertile grains on individual panicles. As the leaves of diseased plants change into yellow in color, carbon assimilation is considered to be less effective than in green leaves. Iodine reaction of diseased leaves was reported to be strong in the morning (Chaimongkol 1971), suggesting inhibited translocation of accumulated carbohydrates in the diseased plants. Such abnormalities would also cause a deleterious influence on the yield.

Relationship between yield and shortening of culm length was also analyzed using data obtained in larger scale paddy fields. When the data from 24 plots of the insecticide application test field in Chapter 4 were used, correlation coefficients between yield and average culm length, between average culm length and disease percentage, and between yield and disease percentage were $r=0.867^{**}$, $r=-0.851^{**}$, and $r=-0.766^{**}$, respectively. Yield damage was thus proved to be highly correlated to shortening of culm length caused by the disease, not only at experimental level, but also field level.

6. Some properties of the virus

Rice Yellow Orange Leaf Virus (YOLV) disease has been reported to be transmitted by 4 kinds of insects, *Nephotettix virescens*, *N. nigropictus*, *Recilia dorsalis*, and a certain species of mealy bug. Among these, *N. virescens* transmitted the virus most effectively (Wathanakul *et al.* 1967c, Wathanakul 1969). The mealy bug was not used in the present experiment, because of difficulty in species identification and in mass rearing.

The percentage of YOLV transmission by *N. virescens* was 53.1% in female adults, 50.6% in male adults, and 70.0% in 4—5th instar nymphs. When virus acquisition source differed, transmissibility remarkably changed. For example, virus was transmitted by 8 out of 30, and in an other case by 72 out of 91 female adults. These differences in virus transmission rate were considered to be caused by different virus concentrations in diseased leaves used for the virus acquisition source. The results showed that the transmissibility of female adults is about the same as that of male adults, or slightly higher, and that of nymphs may be higher than that of adults. These tendencies are in accord with the findings by Wathanakul and others (Wathanakul *et al.* 1967b, 1967c).

N. nigropictus transmitted the virus, but the ability was very low: 2 out of 538 adults and 1 out of 174 nymphs transmitted the virus. *R. dorsalis* also transmitted the virus. The ability was also very low: 8 out of 727. The positive cases were confirmed by back-inoculation using *N. virescens*. When YOLV was transmitted by *N. nigropictus* or *R. dorsalis*, symptoms induced on rice seedlings were not so clear or not so severe as those induced by transmission by *N. virescens*. When the virus was back-inoculated by *N. virescens* from these seedlings with mild or slight symptoms, more distinct and typical symptoms appeared on the back-inoculated seedlings, indicating that the former 2 species transmitted the same kind of virus which was transmitted by *N. virescens*. Milder symptoms may have been caused by a smaller amount of inoculated virus. Mild symptoms were also observed by Lamey when *N. nigropictus* was used (Lamey 1967).

Nymphs of *N. virescens* of all instars transmitted YOLV. The transmission ability appeared to be lower in the 1st and 2nd instar and higher in the 3rd, 4th and 5th instar. Virus retension by nymphs became longer as the instar progressed; retention period was, from the 1st to 5th instar, 1, 3, 3, 4, and 5 days, respectively. The virus retension period in these nymphs was limited by moulting, the result agreeing with the report that Rice Tungro Virus was lost at moulting (Ling 1966). Adults retained the virus for 5 days; most of them lost it after 3 days. The 5th instar nymphs may retain the virus longer than adults, if moulting did not occur.

Virus was isolated from 44 out of 47 rice plants 3 days after inoculation, 36 after 2 days, 10 after 1 day, and 1 after 7 hours. The virus can thus be recovered only 2 days after inoculation in most cases, and this is before symptom appearance. This is considered to be one of the causes of unusually rapid spread of this disease.

A study of the host range of YOLV was reported in Thai in 1966. In this report only wild rice, *Oryza* sp., was reported to have shown clear symptoms among the plants tested, *Brachiaria mutica*, *Chloris barbarta*, *Echinochloa crus-galli*, *Eleusine indica*, *Panicum repens*, *Paspalum conjugatum*, *Leersia hexandra*, and *Oryza* sp. (Wathanakul *et al.* 1967c, 1968, 1970b).

We tested 21 species of Gramineae and 2 species of Cyperaceae in this experiment, but again, only wild rice, *Oryza rufipogon*, showed clear symptoms. The virus could also be transmitted back to rice. *Echinochloa colonum*, *E. crus-galli*, *Leersia hexandra*, *Leptochloa chinensis*, and *L. panicea* showed symptoms of stunting, vein-clearing, interveinal chlorosis, *etc.* The

rice plants back-inoculated from these plants excepting *L. hexandra*, showed dubious symptoms, only which were somewhat similar to YOLV. These symptoms disappeared in several days. Furthermore, virus could not be transmitted from the rice plants further to rice, indicating that the back-inoculation was unsuccessful. *Digitaria adscendens*, *Paspalum distichum*, and *Zoisia japonica* showed no symptoms, but in the back-inoculation test from these, dubious symptoms on rice were also observed.

From these results, wild rice, *Oryza rufipogon*, proved to be a host plant of YOLV. Other species can not be considered as hosts, so far as this experiment is concerned.

Rice Tungro Virus (RTV) was once reported to be infectious to *Eleusine indica*, *Echinochloa colonum*, and *E. crus-galli*. The former, *E. indica*, showed symptoms of yellow specks, *etc.*, while the latter two were symptomless. From these three, RTV was successfully transmitted back to rice. (Wathanakul 1964). A later report from IRRI indicated a wider host range of RTV as follows: *Oryza sativa*, *O. rufipogon*, *O. officinalis*, *O. ridleyi*, *O. barthii*, *Ischaemum rogosum*, *Sorghum vulgare*, *Dactyloctenium aegyptium*, *Eragrostis tenella*, *Leersia hexandra*, *Paspalum scrobiculatum*, *Setaria glauca*, and *Triticum vulgare*. However, except *Oryza* species, only a few plants were infected, such as 3 out of 106 Sorghum plants, 2 out of 55 Triticum plants, *etc.* (IRRI 1968).

Considering these 2 reports on RTV and the present report on YOLV, host ranges of RTV and YOLV might be almost the same, because both the viruses were highly infectious to wild rice, *Oryza rufipogon*, but rarely infectious to gramineous weeds other than *Oryza* spp. Numbers of plants used in the present test may not have been enough to get positive results with weed hosts.

As mentioned above, YOLV was transmitted by *N. virescens* effectively, and by *N. nigropictus* and *Recilia dorsalis* rarely. This is in line with the cases of Rice Tungro Virus (Rivera *et al.* 1965, IRRI 1968) and also similar viruses in Bangladesh (Galvez *et al.* 1969, 1971), in India (Raychauduri *et al.* 1967, John 1968), in Indonesia (Rivera *et al.* 1968), and in Malaysia (Singh 1969, 1971, Ting 1970). Mealy bug was once reported as one of the vectors of YOLV (Wathanakul 1969), but the writers failed to test this insect in the present experiment. Nymph of *N. virescens* lost YOLV at moulting, similar to the cases of Rice Tungro Virus and of Penyakit Merah (Ling 1966, Ting *et al.* 1970). The shape and size of YOLV particles (Saito 1970, Nabheerong *et al.* 1971) are also similar to those of Rice Tungro Virus in the Philippines and Bangladesh (Galvez 1967, 1968, Galvez *et al.* 1971). The host range of YOLV is considered to be almost the same as Rice Tungro Virus and Penyakit Merah, even if differences were observed on some hosts, such as *Echinochloa colonum*, *E. crus-galli*, *Eleusine indica* (IRRI 1968, Ting 1971). Wild rice, *Oryza rufipogon*, is susceptible to YOLV as well as to Rice Tungro Virus. The reactions of rice varieties (Wathanakul *et al.* 1967c) are also almost the same as with Rice Tungro Virus and other similar viruses (Cada 1969, Beachell 1969, Ling 1969, Singh 1969, Rivera *et al.* 1968).

These results show that YOLV is very similar to Rice Tungro Virus. Although there have been no data on serological reactions, chemical composition, *etc.*, it will be safe to conclude, as suggested by the Committee on Nomenclature of Rice Virus Diseases (Ou *et al.* 1969), that YOLV is the same virus, or belongs to virus group, as Rice Tungro Virus.

7. Analysis of the disease prevalence

Four species of the genus *Oryza* are reported to be distributed in Thailand. These are *Oryza meriana* var. *granulata* (=*O. granaulata*), *O. ridleyi*, *O. officinalis*, and *O. rufipogon* (=*O. perennis*, *O. fatua*, *O. sativa f. spontanea*) (Tateoka 1962a,b, 1963). Among these 4

species, *O. meriana* var. *granulata* is found only in mountaineous area of the northern-most region close to Burmese border. *O. ridleyi* grows in moist shaded areas (Tateoka 1963, Akihama *et al.* 1970). *O. officinalis* was recorded as collected around Bankgok (Tateoka 1962a), and we searched it in order to use it for the present experiment. We failed and thought it to be distributed only in certain limited areas. These 3 species, *O. meriana* var. *granulata*, *O. ridleyi*, and *O. officinalis*, are therefore considered to be less important as overseasoning hosts and infection sources for YOLV.

Oryza rufipogon, wild rice, is widely distributed in Thailand and is commonly found in canals, moats, and ponds close to paddy fields (Akihama *et al.* 1970, also our own observation). Even in dry season, *O. rufipogon* is able to survive in irrigation canals where water never dries up, and serves as an overseasoning host of YOLV. In the main rice cropping season, with the development of irrigation facilities, it is becoming of even more importance as one of the foremost infection sources for YOLV.

Air temperature in the hot and dry season is considered to influence overseasoning of vectors. Almost no vectors seem to be able to overseason on hosts grown on dried land during the hot and dry season, when temperature may be 38°C or higher (Chapter 7). Vectors probably overseason on hosts near water, where temperatures are not as high as in dried areas. Wild rice, *Oryza rufipogon*, growing perennially in places where water remains in the dry season is possibly a major overseasoning host of the vectors and of YOLV, as already mentioned. It may be noticed that areas severely infected with YOLV disease fairly correspond to the areas where irrigation facilities are well developed and water remains in the dry season.

YOLV disease inciednce fluctuates according to year. In years of high incidence, the disease prevails in the first half of the rainy season. On the other hand, in years of low incidence, the disease increases gradually and prevalence peak occurs in the last part of the rainy season. Air temperature and rainfall interval in the dry season probably exert great influence on the outbreak of vectors at the beginning of the rainy season, and also on the amount of YOLV overseasoning on host plants. In order to analyze yearly changes of YOLV disease incidence, it is necessary to accumulate more data on disease occurrence, vector population, weather, *etc.*

In the dispersal of YOLV disease in the paddy field in rainy season, a surprisingly small population of vectors causes very heavy infection, as mentioned in Chapter 3, a phenomenon never observed with other rice viruses in the temperate zone. Features of YOLV distinct from other viruses transmitted by leafhoppers are non-persistency in the vector and short latent period in host plants. Also, leafhopper vectors in the tropical zone might be more active than those in the temperate zone. Relationships between disease incidence and vector behavior have been well investigated in the temperate zone, but those information can not be directly applied to epidemiological analysis of YOLV incidence.

It is evident that YOLV disease is difficult to control by chemicals alone, because a very low population of vectors may cause heavy invection. Use of resistant varieties, such as RD-1 and RD-3, is essential. Breeding of resistant varieties adapted to non-irrigated areas, including deep water areas, is desired. Another efficient measure would be to keep canals, moats, and ponds clean from overseasoning hosts of YOLV and vectors, although it is very difficult.

VI. Summary

1) Selected rice varieties were planted at one month intervals from May 1969 to May 1970, and seasonal changes in the incidence of Rice Yellow Orange Leaf Virus (YOLV) disease were surveyed. YOLV disease was most heavily infected in July on plants transplanted in June. As the season advanced, the disease gradually decreased. Plants transplanted in October or later were almost free from the disease. Large population of the vector, *Nephotettix virescens*, were observed in late June to early July, which later decreased. YOLV disease was very severe in 1969, severe in 1970, and very mild in 1971. The peak month also varied: July in 1969, September in 1970, and October in 1971. The disease was not observed in the dry seasons of 1969, 1970, and 1971.

2) The percentage of viruliferous vectors was high in January, February, September, October, and November. In these months the average temperature was between 26—29°C, which was optimum. The percentage of viruliferous vectors was low in March, April, May, June, and December. The average temperature was above optimum (29—31°C) from March to June. The vector population was also extremely low during this period. In December, the temperature was below optimum (23—26°C). The percentage of viruliferous vectors increased gradually from low to high in July and August, the first half of rainy season when average temperature was in the optimum range, 28—29°C.

3) Time of infection with YOLV was surveyed by cultivating rice in the rainy season. The percentage of infected hills in the nursery bed was as follows: in Exp. I, 0%, 5.9%, and 79.4% after 25, 36 and 45 days from sowing, respectively; in Exp. II, 24.8% and 93.2% after 25 and 35 days, respectively. The percentage of diseased hills in the paddy field was higher than the percentage of infected hills at the time of transplanting. Even in the field where only healthy seedlings were transplanted, diseased hills attained 40.5% 37 days after transplanting. The vector population in the nursery bed was about equal in Exp. I and II, but the percentage of viruliferous vectors in the nursery bed in Exp. II was higher than in Exp. I. The vector population in the paddy field in both experiments was very low. The infection rate in the nursery bed was 2.5 to 3.8 times higher than in the paddy field in Exp. I, and 1.9 times higher in Exp. II.

4) The effect of insecticide application for the control of YOLV disease was tested, by using Sevin Granules. The number of adult vectors, *Nephotettix virescens*, collected from 2.25 m^2 at the end of nursery period was 84 adults where no insecticide was applied, and one adult where the insecticide was applied. The number of leafhopper nymphs collected was 1,855 in the unapplied nursery bed and only one in the applied nursery bed. In the paddy field, the percentage of diseased hills and hills showing dubious symptoms was lowest in the plot with 4 applications and highest in the untreated plot. Plant height, culm length and yield showed the highest values in the plot with 4 applications and the lowest values in the untreated plot.

5) The average culm length of YOLV-diseased plants was 31.5% less than that of healthy ones. The average panicle length of diseased plants was 11.4% shorter than that of healthy ones. Compared at the same culm length, the panicle lengths of diseased plants were about equal or sometimes a little longer than those of healthy ones. The grain weight on a panicle averaged 0.79 g in diseased plants and 1.54 g in healthy ones. The grain weight of diseased plants was always lighter than that of healthy ones even at the same culm length. The weight of 1,000 fertile grains (not the conventional 1,000 grains weight) averaged 17.1 g in diseased plants and 19.8 g in healthy ones. The correlation coefficients between yield

and average culm length, between average culm length and disease percentage, and between yield and disease percentage were r=0.867**, r=−0.857**, and r=−0.766**, respectively.

6) The percentage of virus transmission by *Nephotettix virescens* was 53.1% in female adults, 50.6% in male adults, and 70.0% in nymphs. The percentage of virus transmission by *N. nigropictus* was low: 0.4% in adults and 0.6% in nymphs. The percentage of virus transmission by *Recilia dorsalis* adults was also low and was 1.1%.

7) Nymphs of *N. virescens* of every instar transmitted YOLV. In the 1st instar, 7 out of 19 nymphs transmitted the virus, 2nd instar 14 out of 22, 3rd instar 28 out of 36, 4th instar 34 out of 44, and 5th instar 55 out of 80. The longest virus retention period was one day in the 1st instar, 3 days in the 2nd and 3rd instars, 4 days in the 4th instar, and 5 days in the 5th instar. Adults retained the virus for 5 days at maximum. Serial virus transmission ended on the day of moulting, except in 2 cases. The average virus retention period in the 5th instar nymphs would have been longer than that in adults if moulting did not occur during the period.

8) Growth rate of *N. virescens* nymphs was determined under different temperature conditions. At 23—30°C, mortality was almost the same and was 18—26%. At 33°C, it was 48%, and at 38°C, 100%. The nymph period was shortest at 33°C; it became longer at higher and lower temperatures. The nymph period of males was slightly shorter than that of females. The optimum temperature for the growth of nymphs appeared to be 28—30°C.

9) YOLV was inoculated to 10-day-old rice seedlings, variety Taichung Native 1, by using 5 individuals of *N. virescens*, per seedling and then back inoculation test was made by using 5 healthy individuals of the vector per seedling. The vectors, fed on the inoculated seedling just after inoculation, transmitted the virus in one out of 50 cases; those fed after one day transmitted it in 9 cases; after 2 days in 26 cases; after 3 days in 8 cases; and no transmission occurred in 6 cases. The latent period of YOLV in rice seedlings was thus 2 days in most cases.

10) YOLV was inoculated to 21 species of *Gramineae* and 2 species of *Cyperaceae* plants. Wild rice, *Oryza rufipogon*, showed almost the same symptoms as the cultivated rice and virus was successfully back-inoculated to rice. *Echinochloa colonum*, *E. crus-galli*, *Leersia hexandra*, *Leptochloa chinensis*, and *L. panicea* showed dubious symptoms, but virus could not be back-inoculated. The following species showed no symptoms and virus was not back-inoculated: *Brachiaria mutica*, *Chloris barbarta*, *Cynodon dactylon*, *Dactyloctenium aegyptium*, *Digitaria adscendens*, *Eleusine indica*, *Eragrostis tenella*, *Eriochloa annulata*, *Hordeum sativum*, *Imperata cylindrica*, *Panicum repens*, *Paspalum disticum*, *Setaria glauca*, *Triticum vulgare*, *Zoisia japanica*, *Cyperus difformis*, and *Fimbristylis miliacea*.

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