TRANSITORY YELLOWING OF RICE AND ITS TRANSMISSION BY THE LEAFHOPPER *NEPHOTETTIX APICALIS APICALIS* (MOTSCH.)[1]

REN-JONG CHIU, TSING-CHE LO, CHIA-LING PI and MING-HSIUNG CHEN[2]

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Introduction

In a general survey of rice fields made in April 1962, the senior author noted a new disease in several experimental plots at the Kaohsiung District Agricultural Improvement Station in the Pingtung Prefecture. This disease characterized by yellowing of lower leaves and reduced tillering of affected plants was later found to be rather widespread in central and southern Taiwan including the Prefectures of Taichung, Changhua, Yunlin, Chiayi, Tainan and Pingtung. It also occurred in great severity in the second crop of 1963 in the Taitung Prefecture in the eastern Taiwan. The disease is of special interest both for its resemblance in symptom expression to rice suffocating disease and its occurrence in some areas previously reported to suffer loss from the so-called "rice suffocating." As observed since 1961, the new rice disease is mainly a problem of the second rice crop in Taiwan. No substantial loss has been caused in the first crop, although it is found of sporadic occurrence in this season.

Apparently, difficulty in separating by symptoms the new rice disease, termed transitory yellowing in the present paper, from rice suffocating of the type found in the Ilan Prefecture, which has long been known to result directly or indirectly from oxygen deficiency in the paddy soil, had led many rice investigators and extension workers to record transitory yellowing under the

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(2) Plant Pathologist, Joint Commission on Rural Reconstruction, Taipei, Taiwan and Professor and Ressarch Assistants, Department of Plant Patholgy, Provincial Chung Hsing University, Taichung, Taiwan, respectively.

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name of rice suffocating. The recorded acreages of 15,273, 15,548 and 25,635 ha.* for rice suffocating in 1960, 1961 and 1962, respectively, must have been the combined acreages of transitory yellowing and rice suffocating of the Ilan type. The combined acreage for 1963 is 3,870 ha. with 3,376 ha. ascribable to transitory yellowing. This latter figure was obtained after the investigators, who made the estimation, had become aware of the distinction between the two rice maladies of different nature.

It is not known how long transitory yellowing has existed in Taiwan. Viewing from its wide occurrence and its great severity in certain areas in recent years, it is believed that the disease might have existed for a long period befor it was first noticed but incorrectly identified as rice suffocating in 1960 in at least some of those areas in Pingtung reportedly to suffer loss from the latter disease. According to Mr. Y. T. Hsieh** the affected fields designated as rice suffocating since 1960 in Kaoshu (高樹), Fongliao (枋寮) and some other areas in the Pingtung Prefecture could definitely be ascribed to transitory yellowing.

The purpose of this account is to describe the new disease of rice and to present experimental evidence which relates the disease to the feeding on plants by the green rice leafhopper, *Nephotettix apicalis apichlis* (Motsch.). By the vectorship of the leafhoppers, the virus nature of the disease is established.

Methods and Materials

Except in experiments intended to determine varietal reactions, variety Taichung 65 (*japonica* type) was used throughout the study. Seed were sown in 27.5 cm. pots. Transplanting of seedlings singly to 12 cm. pots was made 7–10 days after sowing. In another week the seedlings reached 3–4 leaf stages and were used for inoculation.

All transmission experiments involving the use of insects were conducted with the green rice leafhopper, *Nephotettix apicalis apicalis*. Cultures of the test insect were maintained on rice plants enclosed in wooden cages, 36×36×76 cm^3, which have the top and two lateral sides in 60–mesh netting of wire. In transmission experiments, cages, 10 cm. in diameter and 30 cm. high, constructed by fitting 30–mesh nylon sheeting on wire frame were used most often for confining a single insect or a few insects on test plants. The insects were first placed in the cage towards the closed end, the cage was then

* The recorded acreages for rice suffocating disease are based on mimeographic materials distributed by the Provincial Department of Agriculture & Forestry. The area affected with transitory yellowing in 1963 was estimated by Mr. Y. T. Hsieh.

** Agronomist, Kaohsiung District Agricultural Improvement Station, personal communication.

inserted over the test plant with the open base pressed into the soil. Upon transferring the insect from one plant to another, the base of the cage was worked loose from the soil and the top tilted towards a light source. By blowing breath into the cage, the insect was induced to move from the plant and land on the wall of the cage. The cage was then removed from one plant and placed on another. Less often, test insects feeding for the acquisition of the virus were confined in small stalked cylindrical cages having a narrow slit on bottom for the intrusion by a rice leaf and a small hole on top, when opened by sliding, for introducing and removing insects. The cages, 3.5 cm. in diameter and 16 cm. in length, have wire netting for the walls and metal sheeting for the ends. They can be held in position by their supporting stalk rooted in the pot soil. In using these stalked cages, acquisition feeding was assured on a selected leaf which most recently started exhibiting symptoms.

The greenhouse at the Taiwan Provincial Chung Hsing Uuiversity where the experiments were conducted was not heated in the winter months. However, most of transmission experiments were conducted in other seasons when the greenhouse temperature fluctuated between about 15° to 35°C. We did not exerience any substantial difficulty in maintaining the cultures of the test insect or the virus in the summer months when the temperature rose for a short period at day time to around 38°C despite the providing of a water-evaporation cooling system.

In spite of many precautions, occassional infections were observed in the non-inoculated plants intended as controls in some transmission experiments. Some experiments were rendered invalid by such encountering and will not be included in the present report.

Symptoms

On variety Taichung 65, the symptoms of the transitory yellowing disease are not very distinctive. Diseased plants with lower leaves becoming yellowed give the impression of nutritional disturbance.

Under greenhouse conditions, plants inoculated at 4–leaf stage begin to develop yellowing on the fifth or sixth leaf in about 2–3 weeks after inoculation. Younger leaves further above do not show symptoms until still younger leaves unfold. Color change always starts from the distal portion of a leaf blade. Usually, it is more intense in lower leaves than in upper. No mottling is seen in the discolored leaves in young plants. A typical diseased plant observed one month to six weeks after inoculation will have one or two lower leaves becoming distinctly yellowed (orange buff), the middle leaf producing yellowing only on the distal half and the central one or two young leaves showing no symptoms (Fig. 1). At this time leaves which develop before inoculation and remain symptomless may have dropped from the plant.

Few to many brown rusty flecks or patches may appear on the discolored leaves. However, their diagnostic value seems to be doubtful for discolored leaves devoid of fleckings have also been observed under the winter green house conditions.

Reduced tillering adility is another expression of the disease on this variety (Fig. 2). Plants inoculated at seedling or early tillering stage tiller poorly. An average number of tillers observed in 35 pot-grown diseased plants was 4.0 as compared to 8.2 in healthy ones. The average plant height is also slightly reduced by the disease.

The diseased plants frequently show some degree of recovery under green-house conditions. Following an acute stage of leaf yellowing for about one month or so, infected plants may seem to recover gradually and produce no yellow leaves at later stage of their growth. With the falling away of leaves which previously develop yellowing the diseased plants may then appear normal, a characteristic for which the name "transitory yellowing" is given. Less often, diseased plants towards heading stage may continue to show discolored leaves which usually display some degree of leaf mottling as if the plants suffer from mineral deficiency. Regrowths from diseased plants may or may not develop leaf yellowing. The reasons for such unlike responses among individual plants are not known.

The infected plants have a root system comparable to or only slightly less vigorous than the healthy plants. No other obvious change has been noticed (Fig. 3). This lack of apparent abnormalities in roots would constitute a reliable basis for separating plants affected by transitory yellowing from those by suffocating disease. In the latter case, affected plants invariably have their roots greatly reduced both in number and diameter, and usually become gray or blackened.

Different varieties seem to respond to the disease differently. On Tainan 3, a variety of *japonica* type, the disease produces a near lethal effect. Infected plants develop an indistinct yellowing in the lower leaves which are soon

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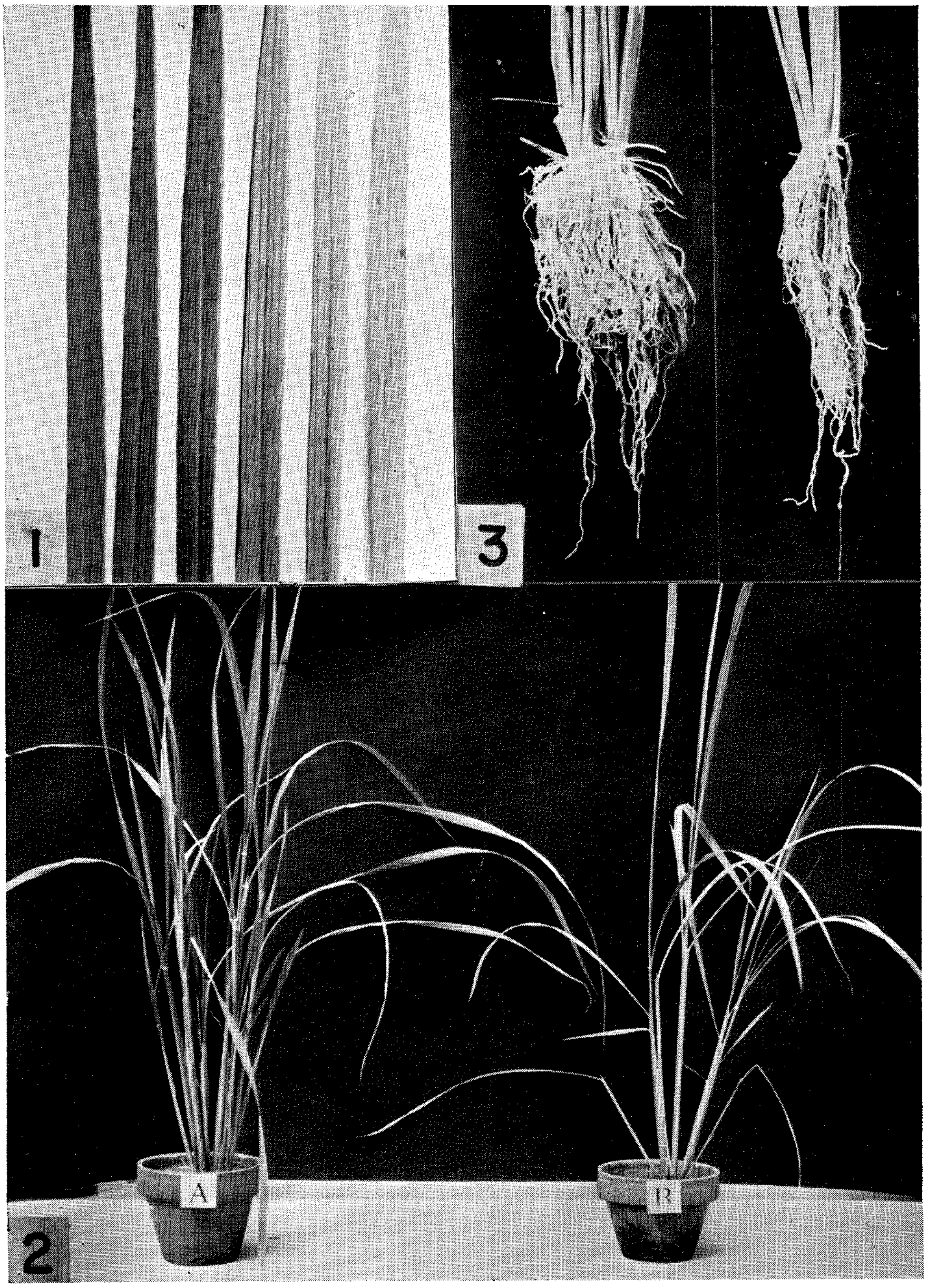
Explanations of Figures

Fig. 1 Six leaves in their natural sequence from a rice plant (variety Taichung 65) infected with transitory yellowing. Three young leaves on left appear normal and green, while three lower ones on right show tangerine orange discoloration.

Fig. 2 Root development in diseased (right) and healthy (left) rice plants of Taichung 65 parallelly grown in the greenhouse. The root system of the diseased plant is less vigorous but shows no apparent abnormalities otherwise.

Fig. 3 A comparison of diseased (right) and healthy (left) plants of variety Taichung 65 showing reduced tillering in the former.


1
3
A
B
2

followed by rolling and drying. Only one or two central leaves of the extremely weakened diseased plants may live. Tillering ability is greatly reduced. On Taichung (native) 1, a variety of *indica* type, which has an excellent tillering ability, the number of tillers in diseased plants is also reduced. Yellowing of leaves is to a lesser degree than on Taichung 65.

Evidence of Natural Spread

Field observations indicate the occurrence of the disease in a rather scattered pattern. Higher incidence is sometimes found in the border rows. Within the same hill which usually contains 4–8 plants, some may be diseased while the remaining are healthy. In less frequent cases, all plants in the hill are affected. This somewhat random manner of disease occurrence both in the fields and within hills suggests an infectious nature.

In an initial attempt to determine whether the disease can be spread by living agents active above ground, experiments were conducted in which pot-grown rice seedlings at about 4–leaf stage were moved out from the greenhouse and placed either under insect cages or uncaged for a one-week period in a field at the Taichung District Agricultural Improvement Station where rice plants were reaching heading stage and the disease incidence was high. The test plants were returned to the greenhouse for symptom expression. Four such experiments each employed 30 plants or more, singly in 4–in. pots. No special caution was taken to prevent the soil organisms from contaminating the pots while they were placed in the diseased field. Before and after the exposure, the test plants were maintained in the greenhouse uncaged.

Of a total of 131 plants used in four experiments, three died of unknown causes before the experiments were concluded. Twenty five produced unmistakable disease symptoms in about four weeks after they had been returned to the greenhouse. Infection occurred to the plants of uncaged groups only. The percentage of infected plants was significantly higher in experiments 1 (28%) and 2 (68%) than in experiments 3 (12%) and 4 (8%). This was consistent with a higher level of disease incidence observed in the field in the second crop of 1962 than in the first crop of 1963. Not a single plant out of forty under cages employed as controls in four experiments was found diseased. These results as presented in Table 1 give clear evidence that the disease is of infectious nature and that an organism active above ground but incapable of intruding the insect cages must aid in its spread. In view of the fact that insect cages used were not fine enough to bar the smaller macroscopic living agents, a conclusion could be reached that the organisms involved in the disease spread would most probably be an insect larger than the tiny mites.

Table 1. *Results of exposing pot-grown rice seedlings in a rice field affected with transitory yellowing*

No. experiment	Period of exposure to natural infection	Plant uncaged			Plant under cage[a]		
		Total	Surviving	Diseased/Healthy	Total	Surviving	Diseased/Healthy
1	Oct. 22–29, 1962	20	18	5/18	10	9	0/9
2	Oct. 29–Nov. 5, 1962	22	22	15/22	10	10	0/10
3	May 9–16, 1963	25	25	3/25	10	10	0/10
4	May 16–23, 1963	24	24	2/24	10	8	0/8

[a] Control plants kept under cages and protected from insects. Test plants kept uncaged.

Incidental Transmission by Leafhoppers in Greenhouse

The first evidence relating the new rice disease to feeding by the green rice leafhopper, *N. apicalis apicalis* (Motsch.), a known vector of the rice yellow dwarf virus[12,13], was accidentally established in greenhouse experiments designed to determine the incubation period of the rice yellow dwarf virus in the vector. From a colony of *N. apicalis apicalis* believed to be free from the test virus, three adults each were taken on August 31 and September 7, 1962 in two separate experiments (Experiments 1 and 2 in Table 2), respectively. After the two groups of test insects had fed on yellow-dwarfed plants for three days and again on healthy plants for two weeks, they were transferred individually to healthy rice seedlings, one insect per seedling. Further serial transfers to healthy seedlings were made each day until the test insects died. By the end of October 1962, symptoms typical of the transitory yellowing, instead of the expected yellow dwarf, were observed in plants of five transferring series involving five test insects. Not a single plant of the last series fed by the sixth insect produced the disease. All the test plants failed to show yellow dwarf symptoms by the time when they were finally discarded. The reason for this remains to be determined.

In one later similar experiment, ten insects from a second colony were employed. They were transferred to a yellow dwarf plant on January 4, 1963 and allowed to feed in group for one day. Then they were maintained in group again on healthy plants until January 31 when they were transferred serially at two-day intervals to healthy plants, one insect per plant. The number of plants that an individual insect fed differed greatly since the test insects lived for different lengths of time. One insect was found to cause transitory yellowing symptoms in all of the 17 plants that it had fed, and another insect in seven out of 16 plants. These diseased plants occurred in a continuous series (Table 2). None of the remaining eight insects did induce symptoms in a total of 70 plants on which they had fed.

Table 2. *Transmissian of transitory yellowing to series of rice plants obtained in serial transferring experiments with individual insects,* ***Nephotettix apicalis apicalis*** *from infective colonies*

Expt. No.	Date of first transfer	Insect No.	Number of plants in serial transfer																								
			1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	Sept. 18, 1962	1	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	+	+	+	+					
		2	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−										
		3	+	+	−	+	+	+	+	+	+	+	+	+	−	−											
2	Sept. 20, 1962	1	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	−							
		2	−	−	−	−	−	−	−	−	−	−	−	−	−												
		3	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−							
3	Jan. 31, 1963	1	−	−	−	−	−																				
		2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+								
		3	−	−	−	−	−	−	−	−	−	−	−														
		4	−	−	−	−	−	−	−	−																	
		5	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+									
		6	−	−	−	−	−	−	−																		
		7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		8	−	−	−	−																					
		9	−	−	−	−	−	−	−																		
		10	−	−	−	−																					

In experiments 1 and 2, serial transfers of a single insect to a new plant were made daily and the test insects were all from the same colony. In experiment 3 insects used were from a second colony different from the first and serial transfers of a single insect were made every other day. [a] Plant numbers in serial transfers; +, infected plant; −, non-infected plant.

It is evident from these results that the disease is associated with feeding by the leafhopper, *N. apicalis apicalis.* That the symptoms are not a result of toxic effects on rice plants exerted by insect feeding can be seen from the experiments to be described in the following sections.

Transmission Experiments

In transmission experiment series, two virus sources were used, the first, G–1, which was used most often was derived from a plant incidentally infected in the greenhouse (Experiment 3 in Table 2) and the second, TD–2, from a pot-grown plant that became infected after an exposure for seven days in a diseased field (Experiment 3 in Table 1).

As the previous experiments had showed that the two existing colonies of *N. apicalis apicalis* might have been contaminated with infective individuals, it became desirable to establish new non-infective colonies before transmission experiments could be attempted. Eggs were located in plants on which adults either from the greenhouse cultures or directly collected from the fields had been previously confined. Nymphs were transferred to healthy seedlings as soon as they hatched from eggs. It is very unlikely that the nymphs had commenced feeding before they were transferred to new healthy plants.

The following transmission experiment was carried out using insects from one of such newly established insect cultures. A large number of non-infective insects were first divided into two groups, one being allowed to feed for two days on a diseased plant and the other on a healthy plant. Thirty insects were taken from each group and serial transfers of three insects per new plant were made at 2–day intervals. The remaining insects of both groups were maintained separately on healthy plants and needed as replacements for those which died on serial transfers. Replacements for the lost insects in the control group were also made from the non-infective colony. Serial transfers were carried through the eighth in each of the 10 series in the control group. In the test group, they were ended at the eighth transferring in five series and earlier in the remaining five because of a loss of the test insects and exhaustion of the reserved stock for replacement. The number of insects per plant was also reduced at last few transfers.

Table 3 summarizes the results of this experiment. The test group involved a total of 67 plants of which 20 plants in 6 series were later found infected. None of the 80 plants in all ten series in the control group developed the disease symptoms. These results clearly demonstrate the need for an access to a diseased plant by test insects before infectivity could be developed in the insects.

Table 3. *Results of serial transferring of* ***Nephotettix apicalis apicalis*** *in a transmission experiment with transitory yellowing virus using 3 insects per plant*

Acquisition feeding[a]	Date of 1st transferring	Insect No.	No. of plants in serial transferring									
			1	2	3	4	5	6	7	8	9	10
2 days (May 14-16, 1963)	May 16, 1963	1	–	–	–	–	–					
		2	–	–	–	+	+					
		3	–	–	–	–	–	–	–	–		
		4	–	–	–	–	+	+	+	+		
		5	–	–	–	–	+	+	+	+		
		6	–	–	–	–	–	+	+	+		
		7	–	–	–	–	–					
		8	–	–	–	–	+	+	+	–		
		9	–	–	–							
		10	–	–	–	+	+	+	+			
Controls —None	May 16, 1963	1	–	–	–	–	–	–	–	–		
		2	–	–	–	–	–	–	–	–		
		3	–	–	–	–	–	–	–	–		
		4	–	–	–	–	–	–	–	–		
		5	–	–	–	–	–	–	–	–		
		6	–	–	–	–	–	–	–	–		
		7	–	–	–	–	–	–	–	–		
		8	–	–	–	–	–	–	–	–		
		9	–	–	–	–	–	–	–	–		
		10	–	–	–	–	–	–	–	–		

[a] Acquisition feeding for two days was allowed on an infected rice plant carrying the virus source, G-1. Serial transfers at two-day intervals were started with 3 insects per plant. Replacements with insects from the reserved group were made for those that died on early transfers. The control insects were transferred in the same manner, except they were not allowed an acquisition feeding previously. +, infected plant; –, non-infected plant.

During March to June 1963 a series of transmission experiments was conducted in which insects after acquisition feeding were tested singly for infectivity on healthy plants. The test groups of insects, usually containing both nymphs and adults, were taken from the non-infective colonies. They were first allowed an acquisition feeding 2 or 3 days and subsequently transferred to series of new healthy plants, one insect per plant. A comparable group from same insect cultures but with no access to diseased plants was always included as control. Parallel serial transfers of both test and control groups were made at 2- or 3-day intervals. Results of two such experiments are presented in Table 4.

Table 4. *Results of serial transferring* ***Nephotettix apicalis apicalis*** *in transmission experiments with transitory yellowing virus using a single insect per plant*

Expt.[a] No.	Acquisition feeding time	Identity and sex of test insect	No. of serialtransfer									
			1	2	3	4	5	6	7	8	9	10
Expt. 1	2 days (Apr. 29-May 1, 1963)	1 (N)	−	−								
		2 (♂)	−									
		3 (N)	−	−	−	−						
		4 (♂)	−	−								
		5 (♂)	−	−	−	+	+	+	+	+	+	+
		6 (N)	−									
		7 (N)	−									
		8 (♂)	−	−	−	−	+	+	+	+	+	+
		9 (♂)	−	−	−	−	−					
		10 (N)	−	−	−							
		11 (♀)	−									
		12 (♀)	−									
		13 (♂)	−	−	−							
		14 (♀)	−	−	−	−						
		15 (N)	−	−	−	−	−	−	−	−	−	
	Controls —None	1 (A)	−	−	−	−	−	−	−	−	−	
		2 (A)	−	−								
		3 (A)	−	−	−	−	−	−	−	−	−	
		4 (A)	−	−								
		5 (A)	−	−	−	−	−	−	−	−	−	
		6 (A)	−	−	−	−	−	−				
		7 (A)	−	−	−	−	−	−	−	−	−	
		8 (A)	−	−								
		9 (A)	−	−								
		10 (A)	−	−								
Expt. 2	2 days (June 10-12, 1963)	1 (♂)	−	−								
		2 (♀)	−	−	+	+	+					
		3 (N)	−									
		4 (N)	−									
		5 (N)	−	−	−	+	+					
		6 (N)	−	+	+	+	+					
		7 (♀)	−	−	−	−	−					
		8 (♂)	−									
		9 (♀)	−	−	−	+	+	+				
		10 (♀)	−		−	−	−	−				
		11 (♀)	−	−								
		12 (N)	−	+	+							
	None	1 (♀)	−	−	−	−	−	−				
		2 (♀)	−	−	−	−	−	−				
		3 (♀)	−	−	−	−	−	−				
		4 (♀)	−	−								
		5 (♂)	−	−	−	−	−					
		6 (♂)	−	−	−	−	−					
		7 (♂)	−	−								
		8 (N)	−	−	−	−	−					
		9 (N)	−	−	−	−	−					
		10 (N)	−	−	−	−	−					

[a] Test insects were allowed to feed for 2 days on infected plants carrying the virns sources, G-1 and TD-2, in experiments 1 and 2, respectively. They were subsequently transferred singly to series of healthy plants at 3-day intervals. The control insects were similarly transferred, except no previous acquisition feeding allowed. +, infected plant; −, non-infected plant; N, nymph; A, adult with its sex not recorded.

Fifteen insects were employed in the test group in experiment 1 (Table 4). Nine died at or before the third transfer. Of those six which lived longer, two transmitted the disease, respectively, to five and six test plants in succession. None of the control group of ten insects became infective. In experiment 2 (Table 4), five insects out of 12 in the test group transmitted the disease to healthy plants on or after the second transfer. None of the ten insects in the control group developed infectivity. The results give further evidences that transitory yellowing is transmissible by certain, but not all, individuals of *N. apicalis apicalis.*

There existed a definite latent period of several days between the commencing of feeding on virus source plant and the appearance of infectivity in the insects. Experiments conducted did not permit an accurate determination of the time length of such period which apparently varied from individual to individual and from experiment to experiment. The minimum possible latent period was estimated in separate cases by assuming that the virus is acquired on the last day of acquisition feeding and transmission is to occur on the first day of inoculation feeding. Similarly, the maximum latent period was estimated by making assumptions that the virus is acquired on the first day of acquisition feeding and that transmission is to occur on the last day of inoculation feeding. In 15 cases in which attempted transmission was positive, the minimum possible latent period ranged from 3 to 24 days and the maximum from 8 to 29 days. The averages were 11 and 16 days, respectively. Evidently, the incubation period for transitory yellowing virus in *N. apicalis apicalis* is much shorter than that for the rice yellow dwarf virus which as determined by Shinkai[12] ranged from 29 to 46 days for the same or related leafhopper. It is very much close to or only slightly shorter than that for the rice dwarf virus. In Fukushi's work, an incubation period exceeding 10 to 40 days and, in one case, prolonged to two months was obtained for the dwarf virus in *N. cincticeps*[2]. In the most cases, it was 30 to 45 days[3]. Shinkai[11] obtained an incubation period ranging from 10 to 58 days for this same virus-vector combination.

Experiments on Other Possible Means of Virus Transmission

In limited experiments, attempts were made to transmit the virus in the following ways: (1) by mechanical inoculation, (2) by the use of seeds from infected plants and (3) by growing plants in soil taken from diseased fields. All these tests gave negative results.

In mechanical transmission tests, either young leaves that did not show yellowing symptom or lower leaves that became yellowed were removed from diseased plants and ground in water or 0.1 M neutral K_2HPO_4 to provide inocula. Dilution was made by adding 4 ml of the diluent to each g. of the leaf tissue. After passing through several layers of cheesecloth, the leaf extracts containing 400-mesh carborundum as abrasive were rubbed over healthy rice seedlings at 4-leaf stage. A total of 118 rice seedlings of the susceptible variety Taichung 65 received inoculation in 3 separate experiments. None developed the disease.

Possibility of seed transmission was tested by planting in the greenhouse seeds that were collected from field grown plants. Rice plants having transitory yellowing symptoms at heading stage were observed in the second crop of 1962 in several experiment fields in the Chiayi Agricultural Experiment Station in Central Taiwan. At harvest, seeds were collected from both diseased and healthy plants of five varieties, namely, Hsinchu 56, Taichung 171, Taipei 306, Nung-shih 20 and Nungshih 25. Twenty plants of each variety were grown from the seeds in both the test and control groups and kept for 45 days after transplanting. By the time the test plants were finally discarded, none in either group developed the disease, a result indicating that seed transmission is not likely. The lack of seed transmission was also obtained in another experiment in which 214 plants of the variety Kaohsiung 10 and 208 plants of the variety Chinan 8 were grown from seeds harvested from diseased plants of the second crop of 1963 from Pingtung area and kindly supplied by Y. T. Hsieh. Observation through 2-month period after transplanting did not reveal any single plant with transitory yellowing symptoms.

In attempting to transmit the disease through soil, infected plants with surrounding soil unremoved were taken from diseased fields and maintained in 27.5-cm. pots in the greenhouse in several occasions. New seedlings were then transplanted to the same pots and grown by the side of the diseased plants. In no case was a spread of the disease observed. In another occasion, 13 healthy seedlings were grown singly in pots that were filled with soil taken from other pots containing infected plants by inoculation. By the time when the test plants were discarded 49 days after transplanting, none showed disease symptoms. Therefore, soil transmission of the new rice virus also seems unlikely.

Discussion

The experimental results as presented above definitely relate the transitory yellowing of rice to feeding by the green rice leafhopper *N. apicalis apicalis.* This relationship could be explained by assuming either that the leafhopper transmits a virus which is the cause of the disease or that the leafhopper is toxicogenic and upon feeding on rice plants is capable of secreting a toxin the effect of which results in the observed symptoms. That the latter is not a likely explanation can be seen from the following: (1) In strictly controlled experiments, only individuals of those insect groups which were previously allowed an access to diseased plants could later develop the property of inducing same disease in the test plants. (2) Regardless of a lymph or adult being used, there existed a latent period between the starting of feeding on diseased plant and the developing of the capacity to induce the same disease on test plants, and (3) In conducting alternative passages between plants and insects, we successfully carried the disease inducing agent twice through rice plants and twice through leafhoppers with a passage through the rice plant alternating a passage through the insect. This should not be expected in the case of a nonmultiplicable toxin often produced by the so-called toxicogenic leafhoppers[1,6]. We therefore are led to conclude that the rice transitory yellowing is a virus disease transmissible by the leafhopper, *N. apicalis apicalis.*

Both nymphs and adults of *N. apicalis apicalis* were able to acquire transitory yellowing virus from the diseased plants. Once rendered infective by an access to virus source plant, most of the leafhoppers retained the virus and were able to transmit it as long as they lived. Daily transfers of some infective insects failed in some cases to transmit on occasional days, thus, a pattern of intermittent transmission resulted. However, this was not seen in experiments in which serial transfers were made at 2–to 3–day intervals. No difference in transmitting efficiency between the male and female adults was apparent from the rathar limited experimental data obtained thus far.

Transitory yellowing is probably closely related to the "Tungro" disease of rice which, according to Rivera and Ou[8], may have long existed in the Philippines under various names. The latter disease transmitted also by *N. apicalis* produces the following major effects on rice plants: (1) yellowing of leaves in diseased plants usually starting from the leaf tip, (2) stunted growth in susceptible varieties, and (3) more or less reduced tillering. All these symptoms are expressed in rice plants infected with transitory yellowing. The Tungro disease resembles transitory yellowing in one further aspect for Rivera and Ou also observed recovery from symptoms in infected plants of the "more resistant varieties". In spite of these similarities, the two viruses may not be identical. We observed very mild yellowing in inoculated seedlings of the variety Taichung (native) 1, while the same variety is shown to respond to the Tungro infection by producing conspicuous color change. Further studies to reveal the vector specificity, the host range, and the length of incubation period in both insect and plant would provide better basis of comparison.

Similarities in symptomatology are also found between transitory yellowing and the orange leaf of rice reported from the Philippines (9). The common effects observed include the reduced tillering and the progressive color change in leaves starting from the lower leaf position to upper and from the leaf tip to base. The latter characteristic distinguishes transitory yellowing, the Tungro disease and the orange leaf disease as a group sharply from other virus diseases on rice transmitted by a leafhopper or a planthopper. This later group includes rice dwarf, yellow dwarf, stripe[13], and Hoja Blanca[5], all of which produce the most conspicuous symptoms in the young leaves. According to Rivera *et al.*[9], the orange leaf is transmitted by the leafhopper, *Inazuma dorsalis* (Motsch.) but not by *N. apicalis*. The vector relationship therefore constitutes at the present a basis for separating the disease from transitory yellowing.

The efficiency of *N. apicalis apicalis* to transmit transitory yellowing seems to be rather low as compared to reported cases of rice dwarf and yellow dwarf transmission by their vector insects. With an acquisition feeding for one day, 100% of the test insects of *N. apicalis* could be rendered infective in the case of yellow dwarf virus[10]. Allowed a same acquisition feeding time, about 70% of insects of *N. cincticeps* developed infectivity in the case of rice dwarf virus[11]. In our experiments, 13 out of 30 insects (or 43%) which lived for two weeks or longer after commencing feeding on the virus source plants developed infectivity, if allowed a 2-day acquisition feeding; and 18 out of 28 insects (or 66%) became infective, if allowed a 7-day acquisition feeding. However, differences in transmitting efficiency between insect cultures of different geographical or hereditory background have been reported for the rice dwarf virus in *N. cincticeps*[4] and for the wound tumor virus and the potato yellow-dwarf virus in *Agalia constricta*[7]. This may possibly be true with *N. apicalis apicalis* in transmitting transitory yellowing. With an acquisition for 24 hours on Tungro diseased plants 67% of test insects of *N. apicalis* of the Philippine origin became transmitters[8], a higher percentage than we have obtained with transitory yellowing in the same vector insect allowed a longer period of acquisition feeding.

The studies on the possibility of the virus to pass transovarially in its vector and on other biological aspects of the relation between the virus and its vector are in progress.

Summary

Transitory yellowing of rice, a new disease affecting seriously the second crop in central and southern Taiwan, was misidentified in previous years as rice suffocating because of their similarities in above ground symptom. Disease plants show leaf yellowing extending from the lower position to upper and from the tip to base. The number of tillers is greatly reduced. The roots of the diseased plants are not much affected or only slightly less vigorous than normal. The infectious nature of the disease was first established by two almost simultaneous findings: (1) by placing of pot-grown plants for 7 days in a diseased field, the disease was induced in some of the plants uncaged at the time of exposure and in none of those caged; (2) feeding by some individuals of the green rice leafhopper *Nephotettix apicalis apicalis* (Mostch.) incidentally induced the disease in successions of rice plants. In transmission experiments which involved serial transfers of the leafhopper singly or in group of 3, transmission was obtained with some but not all of the test insects previously fed for 2 or 3 days on a diseased plant. Inoculated plants developed symptoms in about 2 to 4 weeks after feeding by infective insects. Both nymphs and adults were able to acquire and transmit the virus. Attempted transmission by mechanical inoculation, by planting seed from infected plants and by growing rice seedlings in soil taken from diseased fields was unsuccessful. Transitory yellowing resembles the Tungro disease of rice reported from the Philippines in symptom and in the vector relationship. Varietal reactions to the two diseases are not identical.

水稻黃葉病及黑尾浮塵子傳病試驗[1]

邱人璋 羅清澤 畢家玲 陳明雄[2]

水稻黃葉病（Transitory Yellowing）係一新毒素病，在臺中以南及臺東水稻栽培區發生於第二期水稻上，病徵與水稻窒息病近似，故易致混惑。其發生面積，過去均與窒息病併計爲「水稻窒息病發生面積」。併計之面積民四十九年爲15,273公頃，五十年爲 15,548公頃，五十一年爲 25,635公頃。民五十二年之併計發病面積3,870公頃中，屬於黃葉病者 3,376公頃。四十九年至五十一年三年中黃葉病每年實際發生面積在上述併計面積中究佔多少，則難確知。

黃葉病之主要病徵爲下方葉片變黃，在溫室內稻苗經接種後約二星期，病徵開始出現，四葉期接種之稻苗，其第五葉或第六葉靠近葉尖處，首先變爲暗黃綠色，隨後葉色轉黃益甚，終呈桔黃色。接着上方之葉片亦相繼變黃，但中央二三幼葉，則保持正常綠色。變色葉上或出現稀落之銹色斑點或無之，故銹色斑點不能作爲診斷之依據。病株病部除根羣稍小外，無其他異狀。分蘖數目之減少，爲黃葉病另一顯見之病徵，溫室內接種之病株，35株之平均分蘖數爲4.0，對照之健株分蘖數平均爲8.2。

病株一度出現强烈之病徵後，往往有復健傾向。復健之病株，變色葉已經脫落，留存之葉片均呈綠色，故英文病名以 Transitory Yellowing 稱之。但復健株之分蘖數仍比健株減少。水稻品種不同對黃葉病之反應性，亦不一致。在溫室內，臺中在來一號病徵最輕微；

(1) 此研究係受農復會補助在中興大學進行。
(2) 農復會技正，中興大學教授及研究助理。

臺南三號病株變色葉迅速枯死，僅餘中央一二幼葉，故受害最重。臺中65號之反應性則介在二者之間，變色葉之色澤，鮮艷顯明。

水稻黃葉病之傳染性曾於下列試驗中獲得證實：(1) 溫室內培育之稻苗，移置發病田不加蟲罩經七日者，移回溫室後發病率自百分之八至百分之六十八不等，加蟲罩者移回溫室後均不發病。(2) 在水稻黃萎病 (Yellow dwarf) 傳播試驗中，偶然發現溫室內飼養之黑尾浮塵子 (*Nephotettix apicalis apicalis* (Motsch)) 蟲羣含有傳播黃葉病之個體。三次試驗共取用16隻蟲子，先令取食於黃萎病稻株上，然後個別移飼於健全稻苗上，每隔一至二日，移飼一次 結果各蟲均未傳播黃萎病。但其中 7 隻蟲子在健全稻苗上取食後，引起黃葉病之病徵。

爲期進一步證實黑尾浮塵子傳播黃葉病之能力，隨後試驗所用蟲隻，均取自健全蟲羣。第一次傳播試驗中，供試蟲在病株上取食二日者，分爲十組各三蟲，分組移置於健全稻苗上，每隔二日，各組均移換健苗一次，結果，六組供試蟲能傳播黃葉病於健苗，餘四組則否。對照蟲十組所取食之稻苗，始終健常無病徵。另兩次傳播試驗共用供試蟲27隻，取食於病株上，二日後個別放置於健苗上，每隔三日，移飼一次，其中 7 蟲將黃葉病傳播於健稻上。對照蟲共廿隻，均未在取食之健稻上，引起黃葉病病徵。

上述試驗中，媒介黃葉病之浮塵子自開始在病株上取食時算起，至少須歷三日，多則廿九日方能傳播病害於健稻上。由於黑尾浮塵子傳播黃葉病之結果，推知水稻黃葉病係毒素 (Virus) 所引起而由黑尾浮塵子爲媒介之病害。

在其他傳播方式之試驗中，證知水稻黃葉病非由種子傳播，非由土壤傳播亦非可藉病株液汁而傳播者。

菲律賓發生之水稻 "Tungro" 病，病徵與黃葉病近似。媒介蟲種類亦同。但品種反應性則不盡一致。

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