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RICE RAGGED STUNT DISEASE IN THE PHILIPPINES

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ABSTRACT

Rice ragged stunt, a new virus disease, occurred sporadically in the Philippines in 1977. The symptoms are stunting of plants; appearance of ragged leaves, twisted leaves, and vein-swellings; delay in flowering; production of nodal branches; incomplete emergence of panicles; and panicles bearing mostly unfilled grains. The disease is caused by a polyhedral virus of 50-70 nm, and is transmitted by the brown planthopper *Nilaparvata lugens* (Stål). The virus-vector interaction belongs to the persistent group without transovarial passage. Not only *Oryza sativa* but also *O. latifolia* and *O. nivara* are hosts of the virus. Rice plants can be dually infected with ragged stunt and grassy stunt or with ragged stunt and tungro. Experimental results did not yield a positive response of ragged stunt diseased plants to tetracyclines. The rice ragged stunt virus is likely a member of acanthoviruses.

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RICE RAGGED STUNT DISEASE IN THE PHILIPPINES

The first information of the ragged stunt disease available to us was a telegram addressed to the Director, International Rice Research Institute (IRRI), from Mr. Mariano Tirona, Provincial Agriculturist of North Cotabato, Mindanao, Philippines, 14 January 1977, which carried this message:

> "URGENTLY REQUESTING YOUR PLANT PATHOLOGIST CONDUCT IMMEDIATE INVESTIGATION PRESENT RICE MALADY WHICH MAY ENDANGER M-99 RICE PROGRAM PHASE EIGHT NORTH COTABATO STOP REQUEST MADE DUE LACK LOCAL FACILITIES FOR PROPER IDENTIFICATION STOP FINDINGS AND OBSERVATIONS IR26 CMA IR36 CMA AND IR38 VARIETIES PLANTED LATE SEPTEMBER AND OCTOBER UNDER PHASE SEVEN HAD GOOD GROWTH BUT FAILURE TO EXCISE BY PANICLES AT HEADING STAGE ESPECIALLY TILLERS WHICH ARE MUCH SHORTER IN HEIGHT THAN MOTHER PLANTS STOP MALADY SUSPECTED AS DELAYED EXPRESSION OF TUNGRO VIRUS DISEASE WHICH NEEDS FURTHER SCIENTIFIC STUDIES THROUGH INOCULATION TO DIFFERENTIAL VARIETIES FOR PROPER IDENTIFICATION THROUGH IRRI FACILITIES AND EXPERTISE STOP PLANTS AFFECTED PRODUCE ZERO TO FIFTY CAVANS DEPENDING SERIOUSNESS OF DISEASE STOP FURTHER OBSERVATION ARE PRESENCE OF SHEATH BLIGHT ON LEAF SHEATH HOWEVER OBSERVATION IR32 SLIGHTLY INFECTED BY BACTERIAL LEAF BLIGHT STOP PRESENT AREA PLANTED TO ABOUT SIXTY PERCENT IR36 AND FORTY PERCENT IR32"

Similar telegrams were addressed to the Bureau of Agricultural Extension and to the National Food and Agriculture Council of the Republic of the Philippines. In response, rice pathologists of the Philippine Bureau of Plant Industry and the University of the Philippines at Los Baños went to North Cotabato to observe the disease. We were unable to make the trip but we received specimens of the diseased plants.

The diseased plants from North Cotabato were at heading stage and showed abnormalities such as short and twisted flag leaves; incomplete emergence of panicles; excessive nodal branches and nodal panicles; and panicles bearing mostly unfilled grains. Although the disease was then not familiar to us, we could definitely say it was due to neither the late infection of rice tungro disease nor the delayed expression of tungro infection because its symptoms differed distinctly from those of rice tungro disease, and we were unable to transmit the disease to rice seedlings by using the tungro vector *Nephotettix virescens*.

Mr. Tirona deserved all credit for drawing scientists' attention to the disease although several scientists claimed, most of them verbally, that they had seen the disease in the Philippines before 1977. No information about the disease was published before January 1977.

In February 1977, we observed the disease in rice fields at IRRI and later in farmers' fields in Luzon, Negros Occidental, and Mindanao. In most cases, only a few diseased plants scattered over rice fields. In severe cases, however, more than 90% of rice hills in a field were diseased, and yield was totally lost.

Because the disease was definitely not the late infection of rice tungro, and symptoms of the disease were stunting of rice plants and appearance of ragged leaves, we named the disease *rice ragged stunt* on 6 April 1977. However, we later learned the disease was also being called *infectious gall* (de la Cruz 1977) and *kerdil hampa* (Hibino et al. 1977), which literally translated, means stunt empty.

This paper reports the symptomatology, chemical analysis, causal agent, transmission, host range, dual infection, and antibiotic treatment of the ragged stunt disease from studies of materials either directly collected from rice fields or artificially inoculated in the greenhouse. The studies were at IRRI and at the University of Hokkaido, Sapporo, Japan.

SYMPTOMATOLOGY

The symptoms of ragged stunt disease are quite complex, and vary according to stage of plant growth. They include stunting of plants; appearance of ragged leaves, twisted leaves, and vein-swellings; delay in flowering; production of nodal branches; incomplete emergence of panicles; and panicles bearing mostly unfilled grains. However, some features of the diseased plants do not differ strikingly from those of the healthy ones. For instance, the color of diseased plants does not deviate markedly from that of healthy ones. The difference in number of tillers between healthy and diseased plants is not consistent (Table 1), but at the time of maturity of the healthy plants, the diseased plants have more tillers than the healthy plants in pot experiments (Table 2).

Table 1. Growth of healthy and ragged stunt diseased plants of Taichung Native 1 in pots.

Plant	Tillers (no.)				Plant height (cm)			
	0[a]	1[a]	2[a]	3[a]	0[a]	1[a]	2[a]	3[a]
Healthy	1.0	1.9	8.8	8.3	11.8	64.5	75.4	79.8
Diseased	1.0	3.2	8.1	6.6	11.5	38.8	47.0	52.3
LSD (5%)	-	0.7	2.3	2.3	1.6	11.2	13.9	12.9

[a]Months after inoculation.

Table 2. Comparison of healthy and ragged stunt diseased plants of Taichung Native 1 in pots at maturity. Diseased plants were inoculated at seedling stage.

Plant	Tillers (no.)	Nodal branches (no.)	Panicles (no.)	Filled grains (no.)	Unfilled grains (no.)
Healthy	4	0	3	223	54
Diseased	11	33	18	52	474
LSD (1%)	5	7	4	154	114

Stunting

The diseased plants are stunted to various degrees at all growth stages (Fig. 1A). Stunting is the only symptom before the heading stage that is observable from distance. In pot experiments, artificially inoculated plants of Taichung Native 1 (TN1) were only 60 to 65% as tall as the healthy plants during all growth stages (Table 1). The other symptoms are clear only when diseased plants are closely examined.

Ragged leaf

At early growth, the predominant symptom is the appearance of ragged leaves which are leaves with irregularly edged portions (Fig. 1B, 1C). The irregular edge consists of 1 to 17 or more breakings and the edge between or near the breakings. The breakings appear as notches or indentations, and can be observed even before the leaf blades unroll. The depth of the breakings varies but never crosses the midrib; the distance between two breakings varies from 0.2 to 3 cm. Occasionally, the breakings are at a similar distance, resulting in an evenly serrated leaf. The length of the ragged edge varies from 0.1 to about 20 cm, or from less than 1% to about 70% of the length of the leaf blade, including a chlorotic edge beyond the extreme notches at both ends. The ragged portion is generally chlorotic, and the white color may later become yellow or brownish yellow. The chlorotic edge, particularly between two notches, may eventually disintegrate.

The ragged edges occur more on leaf blades and less on leaf sheaths (Table 3). The ragged edges that appear on leaf blades are either on one side or on both sides of the leaf blades, and at different portions of the leaf blades. All of infected rice varieties and lines examined had ragged leaves, but about 86% of diseased hills of various growth stages had ragged leaves. The ragged leaves range from 0 to 34 per hill, or from 0 to 5 per tiller, and gradually become fewer as the plants grow older.

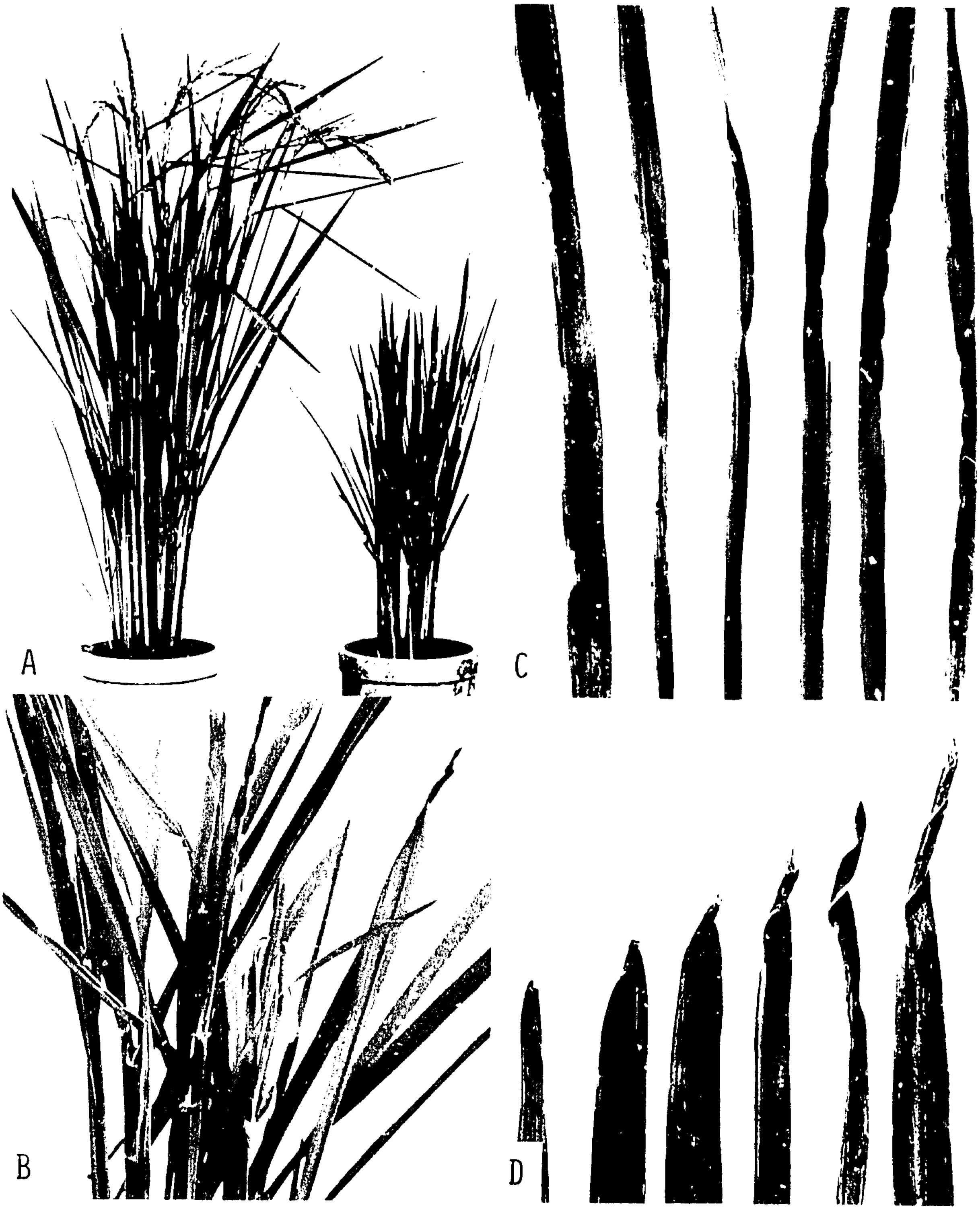

Fig. 1. Symptoms of rice ragged stunt disease.
A. Healthy (left) and infected (right) plants.
B. Closeup of a diseased plant showing ragged and twisted leaves.
C. Ragged leaves.
D. Twisted leaves.

Table 3. Aspects of ragged edges on leaves and ragged leaves on rice plants infected with ragged stunt disease.

Examined (no.)	Designation	Quantity
	Ragged edge	
1359	Number of breakings/edge (range)	1-17
179	Distance between two breakings (range in cm)	0.2-3
439	Length (range in cm)	0.1-20
439	As percent of the length of leaf blade (%)	<1-70
	Ragged edges on	
513	Leaf blades (%)	96
513	Leaf sheaths (%)	4
	Ragged edges on	
495	One side of leaf blades (%)	92
495	Both sides of leaf blades (%)	8
	Ragged edges at	
932	Upper portion of leaf blades (%)	24
932	Middle portion of leaf blades (%)	59
932	Lower portion of leaf blades (%)	17
	Ragged leaves	
47	Number/hill (range)	0-34
640	Number/tiller (range)	0-5
	Having ragged leaves	
243	Rice varieties and lines (%)	100
1486	Rice hills (%)	86
640	Tillers within infected hills (%)	56

Twisted leaf

Another symptom of ragged stunt is twisting of leaves (Fig. 1B, 1D). The twisting often occurs at the top portion of leaf blades. It may wind one or more turns, resulting in a spiral shape. The twisting is caused by the alternate uneven growth of the leaf blade on both sides of the midrib. Almost all varieties and lines examined had twisted leaves. About 30% of the tillers within infected hills had twisted leaves, which varied from 0 to 10 per hill, or 0 to 3 per tiller.

Vein-swelling

Another symptom of the disease is an outward growth of vein tissue, resulting in swollen veins. The technical term for such malformation is histoid enation (Bos 1970) or virocecidium (Blattný 1961), but we prefer to call it *vein-swelling* because the term is specific, descriptive, and understandable. The vein-swellings (Fig. 2A) are due to the proliferation (hyperplasia) of phloem cells in vascular bundles (Fig. 2B).

The vein-swellings vary from about 1 mm to more than 10 cm in length; from 0.2 to about 1 mm in width; and from 0.1 to about 1 mm in height. The number of vein-swellings varies among tillers and varieties, and ranges from 0 to 74 per tiller, or from 0 to 42 per leaf, including both on the leaf blade and on the leaf sheath. The color of vein-swellings varies, about 82% are pale yellow or white; 2% light brown; 6% dark brown; and 10% a combination of such colors (a portion being pale yellow; the other, brown). The color seems to remain unchanged until the leaf dies (Table 4).

The vein-swellings occur on the outer surface of leaf sheaths or the lower surface of leaf blades. None have been observed on the opposite sides, possibly because the vein-swellings result from the proliferation of phloem cells, which are at an abaxial position to the rice leaf.

The vein-swellings appear on different parts of rice plants, but more on leaf sheaths (Table 4). About 90% of those on leaf sheaths are on the upper half. Most appear near the collar. All naturally infected rice varieties and lines examined had vein-swellings during the later tillering and after. The percentage of tillers with vein-swellings seemed to increase as the plants grew older.

Delayed flowering, nodal branches, incomplete panicle emergence, and unfilled grains

At booting stage, flag leaves of the diseased plants are short and often twisted, malformed, or ragged (Fig. 2C). Diseased plants often flower late, and panicle emergence is often incomplete (Fig. 2C). Tillers often generate *nodal branches* which are secondary or tertiary tillers produced at upper nodes of a tiller (Fig. 2D). All the 411 rice varieties and lines examined developed nodal branches. The number of nodal branches varied from 0 to 11 per tiller. It increased as the plant grew older. The nodal branches often bear small panicles -- *nodal panicles* (Fig. 2D). The diseased plants have more panicles and spikelets than the healthy ones (Table 2). Their spikelets are often not discolored. The number of filled grains of the diseased plants is greatly reduced (Table 2). In the field, usually no grains or only a few filled grains can be harvested from a diseased hill.

Systemic infection

The ragged stunt disease is systemic. When a diseased plant is ratooned, vein-swellings are the only symptom that can be observed in the early regenerated growth; other symptoms appear at booting stage of the plant. The diseased plants can survive long after flowering -- more than 6 months in the IRRI greenhouse.

Fig. 2. Symptoms of rice ragged stunt disease.
A. Vein-swellings.
B. The cause of vein-swelling — proliferation of phloem cells.
C. Distortion of flag leaves and incomplete emergence of panicles.
D. Nodal branches and nodal panicles.

Table 4. Aspects of vein-swellings on leaves and culms of rice plants infected with ragged stunt disease.

Examined (no.)	Designation	Quantity
	Size of vein-swellings	
646	Length (range in mm)	1->100
646	Width (range in mm)	0.2-1
646	Height (range in mm)	0.1-1
	Color of vein-swellings	
1099	Pale yellow or white (%)	82
1099	Light brown (%)	2
1099	Dark brown (%)	6
1099	Combination (%)	10
	Vein-swellings	
952	Number/tiller (range)	0-74
419	Number/leaf (range)	0-42
	Vein-swellings on	
1006	Leaf blades (%)	21
1006	Leaf sheaths (%)	72
1006	Culms (%)	7
	Vein-swellings at	
37	Upper half of leaf blades (%)	40
37	Lower half of leaf blades (%)	60
	Vein-swellings at	
56	Upper half of leaf sheaths (%)	90
56	Lower half of leaf sheaths (%)	10
	Having vein-swellings	
66	Rice varieties and lines (%)	100
279	Rice hills (%)	96
952	Tillers within infected hills (%)	67

Inoculated seedlings

When young rice seedlings are inoculated, the first noticeable symptom is stunting, followed by the appearance of ragged leaves and then the other symptoms. The vein-swellings appear on both leaf sheaths and leaf blades of artificially infected plants about 3 weeks after inoculation.

CHEMICAL ANALYSIS

Leaves of both healthy and ragged stunt diseased plants of three rice lines -- IR2061-465-1-5-5, IR3839-1, and IR4427-115-3-3-3-3 -- collected from fields were chemically analyzed for contents of nitrogen, phosphorus, potassium, calcium, magnesium, iron, copper, manganese, and zinc. The element contents of both healthy and diseased plants were between the critical levels of deficiency and toxicity given by Tanaka and Yoshida (1970). Differences in analyzed elements between the healthy and diseased plants were not statistically significant.

CAUSAL AGENT

The nature of the causal agent of the ragged stunt disease was studied with an electron microscope and a bioassay method at the University of Hokkaido in collaboration with Dr. E. Shikata and his associates.

Electron micrographs (Fig. 3) showed polyhedral particles of 50-70 nm in dip preparations and also in ultrathin sections of rice plants naturally and artificially infected in the Philippines. The particles were abundant in phloem cells and in cells of the vein-swellings (Shikata et al. 1977).

The infectivity of rice ragged stunt virus was determined by the injection method. Fresh leaves of diseased plants of IR3839-1, infected in the Philippines, were macerated in a buffer solution and centrifuged. The supernate was injected through a fine glass capillary into the body of the

Fig. 3. Electron micrographs show virus particles in a dip preparation (left) and in a phloem cell of an ultrathin section (right) of a rice plant infected with ragged stunt disease (from Shikata et al. 1977).

brown planthopper *Nilaparvata lugens* (Stål), the colony of which had been reared for 4 years in Sapporo, Japan, where ragged stunt disease has not been observed to occur naturally. Twenty-one of 40 insects that received the supernate injection survived for 2 weeks. They were used to inoculate seedlings of the rice variety Mihonishiki at 3 insects/seedling for an inoculation access time of 3 days. The same insects were then transferred to inoculate another set of seedlings for another 3 days. All inoculated seedlings developed symptoms of the disease, indicating that the injected insects became infective (Senboku et al. 1978a).

TRANSMISSION

The manner of transmission of the ragged stunt disease was investigated at IRRI.

Mechanical transmission

Rice seedlings of IR3839-1, a line susceptible to the disease, were manually inoculated by rubbing leaf blades with the sap of diseased plants, using carborundum as an abrasive. Other seedlings were inoculated by the pin-prick method. None of the 96 inoculated seedlings developed disease symptoms, indicating that transmission of the disease by mechanical means was unsuccessful.

Soil transmission

To determine transmission of the disease through soil (actually, by soil-inhabiting microorganisms), soil near diseased plants and stubble of diseased plants were collected from paddy fields, and placed in trays. Healthy rice seedlings were immediately grown in the trays, both with and without the stubble of diseased plants in the center of the trays. The stubble was free of brown planthoppers. The seedlings were grown until maturity. None of 204 seedlings became infected although disease symptoms developed on the regenerated growth of the stubble in the center of the trays. The disease was not transmitted through soil even in the presence of stubble of diseased plants.

Seed transmission

Mature seeds were collected from diseased plants and germinated. None of 633 seedlings showed symptoms of the disease. Some were transplanted and grown in screen cages until maturity. They remained healthy. Hence, no evidence was obtained to demonstrate the transmission of ragged stunt disease through rice seed.

Transmission by the brown planthopper

Insects with sucking mouth parts that appeared on diseased plants in paddy fields were captured, and used to inoculate rice seedlings in the greenhouse. Only seedlings inoculated by the brown planthoppers (Fig. 4) became infected. Hence, the brown planthopper is the only known vector of the disease.

The ability of the brown planthopper to transmit rice ragged stunt disease to seedlings of IR3839-1 was tested by daily serial transmission after acquisition access times of 2, 3, 4, or more days until the death of the insect. The tests were repeated, and involved 1,625 insects and 28,753 inoculated seedlings, including 1,906 seedlings that died before symptoms developed.

About 40% of the tested brown planthoppers transmitted the disease (Table 5). In a single test with a small number of insects, from 14 to 76% of the brown planthoppers were active transmitters. Both nymphs and adults transmitted the disease. There were no striking differences in percentage of active transmitters between female and male adults (46 versus 42%), and between brachypterous and macropterous forms (42 versus 48%). A few insects became infective immediately after acquisition feeding, while others remained

Fig. 4. Brown planthoppers, *Nilaparvata lugens* (Stål), the vector of rice ragged stunt disease.

Table 5. Transmission data of rice ragged stunt disease by *Nilaparvata lugens*.

Insects tested (no.)	Designation	Range	Mean
1625	Active transmitters (%)	14-76	40
256	Nymphs	0-100	19
1369	Adults	14-82	44
714	Females	15-100	46
655	Males	6-91	42
940	Brachypterous form	16-100	42
429	Macropterous form	0-100	48
500	Latent period (days)	2-33	9
500	As percent of life span (%)	6-96	40
655	Infected seedlings/infective insect (no.)	1-22	4
48	Nymphs	1-8	2
607	Adults	1-22	4
330	Females	1-21	5
277	Males	1-22	4
399	Brachypterous form	1-22	5
208	Macropterous form	1-17	4
500	Retention period (days)	3-35	15
500	As percent of life span (%)	13-100	69
655	Disease-transmitting days (%)	3-100	41
655	Nontransmitting period (days)	0-30	7
655	As percent of life span (%)	0-83	30
165	Congenitally infective insects (no.)	0-0	0

latent for as long as 33 days. The average latent period was 8.6 days. The insects remained infective after molting, indicating that the virus passage is transstadial. The highest number of seedlings that an insect infected during its life span was 22. However, 33% of the infective insects infected only one seedling during their life spans. On average, an infective insect infected about 4 seedlings. The highest percentage of infective insects transmitted the disease 9 days after acquisition feeding. The daily transmission pattern was generally intermittent. The retention period ranged from 3 to 35 days after acquisition feeding, averaging 15 days. The disease-transmitting days varied from 3 to 100% (averaging 41%) of the period from the time that an insect became infective until its death. As the infective insects aged, their transmitting activity decreased. The period between the last successful transmission and the insect's death ranged from 0 to 30 days, averaging 7 days.

The transovarial passage of the virus was determined by examining the infectivity of the progeny of viruliferous insects. The progeny were obtained by rearing the viruliferous brown planthoppers on *Monochoria vaginalis*, a plant species that is assumed a nonhost of ragged stunt virus but is a temporary host of the brown planthopper (Ling and Aguiero 1970). The newly hatched nymphs were used to inoculate rice seedlings of TN1 at 1 insect/seedling per day for 20 consecutive days except when insects died earlier. None of 2,733 seedlings inoculated by 165 progeny insects became infected, indicating the absence of congenitally infective insects.

Consequently, the interaction of ragged stunt virus and the brown planthopper classifies the virus in the persistent group without transovarial passage.

Transmission by the brown planthopper in Japan

The transmission of rice ragged stunt disease by a brown planthopper colony that had been reared on rice plants in a greenhouse in Sapporo, Japan, for 4 years was studied in collaboration with Japanese scientists at the University of Hokkaido. After 1-day acquisition access time on diseased plants of the rice line IR3839-1, which had been infected in the Philippines, the infectivity of 50 brown planthoppers was tested by daily serial transmission to 1,270 seedlings of the variety Mihonishiki for 40 consecutive days except when insects died earlier. The experiment was carried out in a temperature-controlled greenhouse (25-28°C).

Twenty-eight percent of the tested brown planthoppers, including both female and male adults, and both brachypterous and macropterous forms, were active transmitters. The latent period ranged from 5 to 11 days after acquisition feeding, averaging 8.6 days. The infective insects infected from 1 to 31 or more seedlings during their life spans. The retention period ranged from 9 to 41 or more days after acquisition feeding (some insects did not die on the last day of the transmission test, 41 days after acquisition feeding). The disease-transmitting days from the time that the insect became infective until the last day of transmission test ranged from 33 to 100%, averaging 81% (Senboku et al. 1978b).

The brown planthoppers in Japan transmits rice ragged stunt disease. This confirms that the brown planthopper is the vector of the disease because the positive transmission in Japan, where there is no natural occurrence of the disease, could not have been due to comtamination.

Although the percentage of active transmitters and the latent period of the brown planthoppers in Japan and those in the Philippines were similar, the brown planthoppers in Japan infected more rice seedlings during its life span, gave a higher percentage of disease-transmitting days, and had a longer retention period than the brown planthopper tested at IRRI. The reason could be the differences in either the ecotypes of the insects or the conditions for the transmission test, or both. However, we speculate that the difference in transmissive ability between the brown planthopper in Japan and those in the Philippines was primarily due to the difference in the conditions during the transmission test. At IRRI, the transmission tests were done in a greenhouse where the temperature was much higher than that in Japan. The effect of temperature was substantiated by the fact that life spans of the brown planthoppers used for the transmission tests in the Philippines averaged about 22 days, whereas those of the 50 brown planthoppers used for the test in Japan averaged longer than 32 days. The insects with longer life span should have a greater chance to infect more rice seedlings and a longer time for retaining the virus. Temperature affects the insect's life span. At least, it does in the case of *N. virescens* (Ling and Tiongco 1975).

Transmission by biotypes of the brown planthopper

The ability of different biotypes of the brown planthopper to transmit rice ragged stunt disease was studied by daily serial transmission to IR3839-1 seedlings after an acquisition access time of 2 or 4 days. The study included three biotypes from Department of Entomology, IRRI, that differ in ability to attack rice varieties carrying different resistance genes to the brown planthopper (IRRI 1977), and our colony of the brown planthoppers that had been reared on TN1 in the greenhouse for several years but whose ability to attack different rice varieties had not been determined. The test involved 985 insects and 18,477 inoculated seedlings, including 1,382 seedlings that died before symptoms developed.

The brown planthopper biotypes did not differ significantly in percentage of active transmitters, latent period, number of infected seedlings per insect, retention period, or percentage of disease-transmitting days of the period from the time that an insect became infective to its death (Table 6). All the tested biotypes showed transstadial passage and were similar in daily serial transmission (Fig. 5). Consequently, the tested biotypes have similar ability to transmit rice ragged stunt disease.

Table 6. Comparison of transmission of rice ragged stunt disease by different biotypes of *Nilaparvata lugens*.

Biotype	Active transmitters (%)	Latent period (days)	Infected seedlings (no./insect)	Retention period (days)	Disease-transmitting days (%)
1	41	9	5	16	37
2	34	11	4	16	36
3	41	8	4	14	39
Undetermined	37	8	5	14	45
Test of significance	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = no significant difference among biotypes.

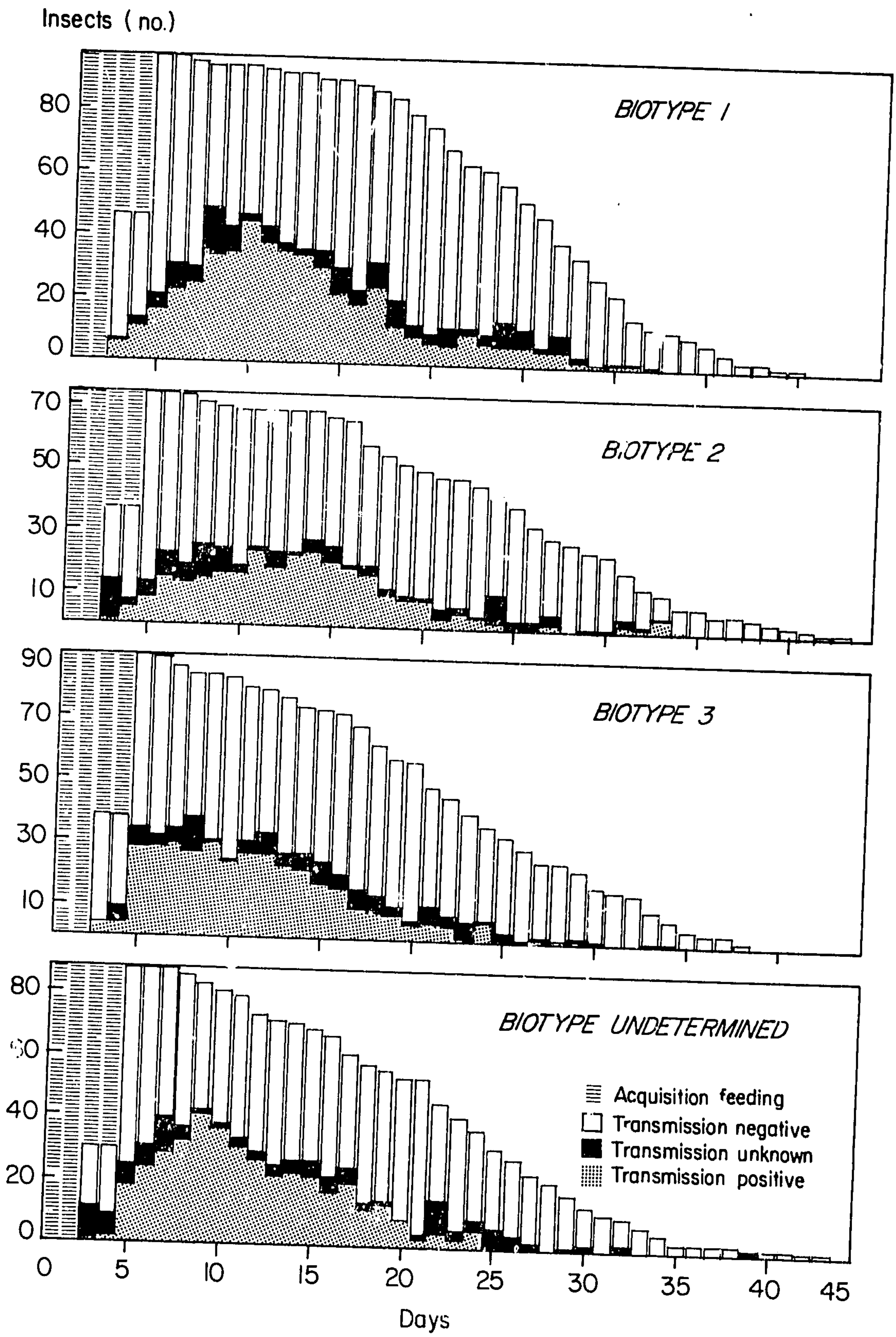

Fig. 5. Daily serial transmission of rice ragged stunt disease by infective *Nilaparvata lugens* biotypes.

Source for transmission

Diseased rice plants of different varieties, from different fields and different localities were collected for the transmission study. The transmission results were similar. There was also no difference between naturally and artificially infected plants.

HOST RANGE

Plants of several species were inoculated by viruliferous brown planthoppers of rice ragged stunt disease in the greenhouse. Among *Oryza* species, *O. sativa*, *O. latifolia*, and *O. nivara* were infected with ragged stunt. The infected plants of *O. latifolia* (Fig. 6A, 6B) and *O. nivara* (Fig. 6C) showed stunting, ragged leaves, vein-swellings, and nodal branches, similar to those on *O. sativa*. Plants of *O. latifolia* were also infected naturally in the field.

Brown planthoppers recovered the virus from the diseased plants of *O. latifolia* and *O. nivara* and transmitted ragged stunt successfully to rice seedlings. Consequently, *O. sativa* is not the only host of the rice ragged stunt virus.

DUAL INFECTION

In the Philippines, besides the newly discovered ragged stunt disease, there are four known virus and virus-like diseases of rice plants -- grassy stunt (Rivera et al. 1966; Ling et al. 1970), orange leaf (Rivera et al. 1963), tungro (Rivera and Ou 1965), and yellow dwarf (Palomar and Rivera 1967). Orange leaf and yellow dwarf are of minor importance (Ling 1972). Rice plants can be dually infected with tungro and grassy stunt diseases (IRRI 1972). Therefore, dual infection of ragged stunt and grassy stunt or of ragged stunt and tungro was studied by inoculating seedlings of TN1 with respective viruliferous insects in the greenhouse.

Rice plants were dually infected with ragged stunt and grassy stunt (Fig. 7A, 7B). The symptoms of the dually infected plants included both the symptoms of ragged stunt (ragged leaves and twisted leaves) and those of grassy stunt disease (severe stunting, excessive tillering, and narrow leaf blades). Plants were much more stunted, particularly at the late growth stage, when dually infected than when infected with one of the two diseases.

Rice plants were also dually infected with ragged stunt and tungro (Fig. 7C). The infected plants showed symptoms of stunting; appearance of ragged leaves, a symptom of ragged stunt; and yellowing of leaves, a symptom of tungro.

Fig. 6. *Oryza* spp. infected with ragged stunt disease.
A. An infected *O. latifolia* plant.
B. Ragged leaves of *O. latifolia*.
C. Healthy (left) and infected (right) plants of *O. nivara*.

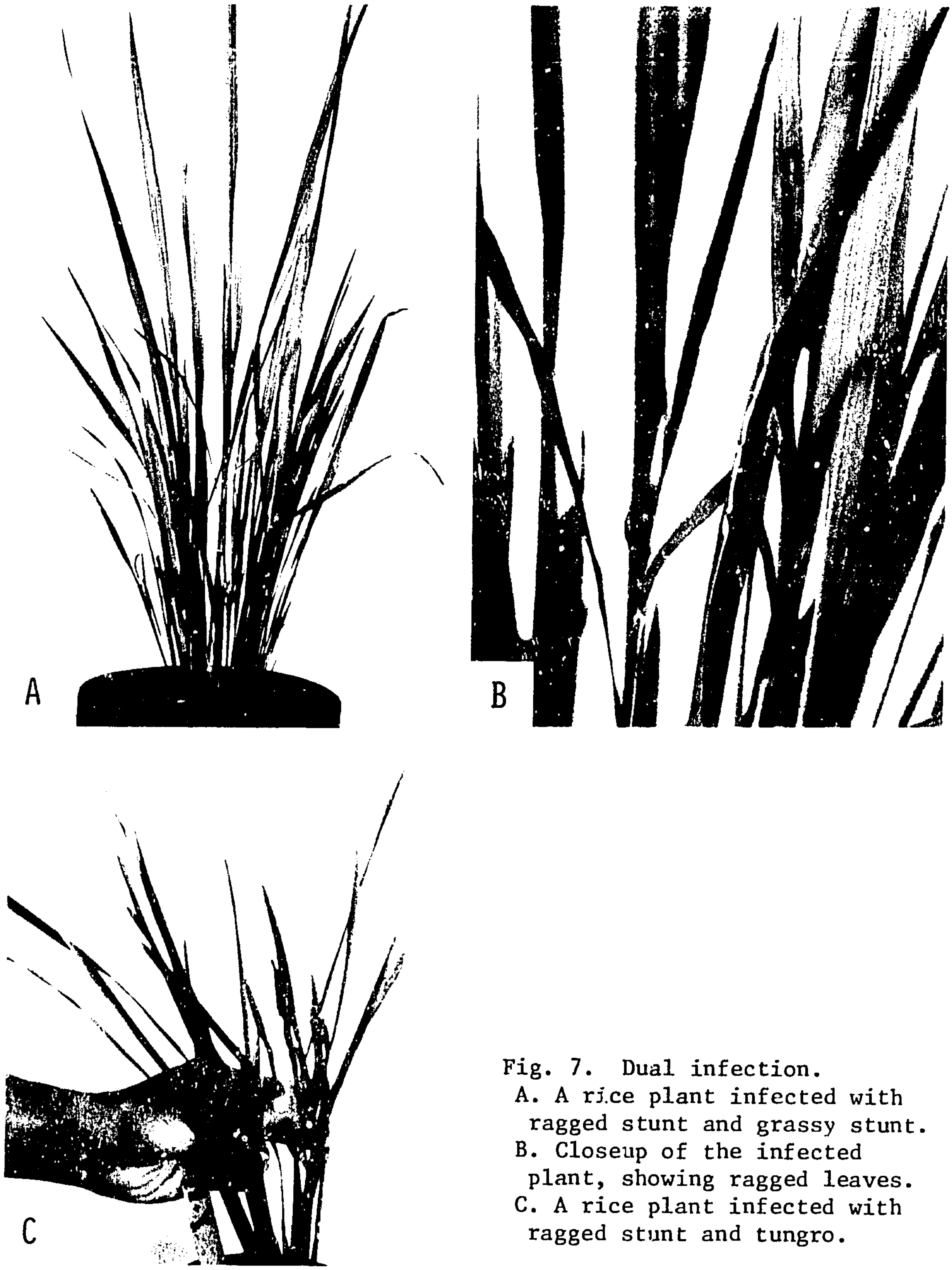

Fig. 7. Dual infection.
A. A rice plant infected with ragged stunt and grassy stunt.
B. Closeup of the infected plant, showing ragged leaves.
C. A rice plant infected with ragged stunt and tungro.

The dual infection indicates the absence of cross protection either between ragged stunt and grassy stunt or between ragged stunt and tungro. Hence, the causal agent of ragged stunt is not closely related to the causal agent of grassy stunt or tungro.

ANTIBIOTIC TREATMENT

Tetracyclines reportedly suppress symptoms of plant diseases caused by mycoplasmas (Whitcomb and Davis 1970), but they have no effect on diseases caused by viruses.

Rice plants of IR29 and IR30, naturally infected with ragged stunt, were transplanted into nine groups of pots at three hills per group and treated with two tetracycline compounds, oxytetracycline and tetracycline hydrochloride. Two concentrations of each compound -- 15 and 60 ppm of oxytetracycline, and 50 and 200 ppm of tetracycline hydrochloride -- were sprayed on the diseased plants either once a week for 11 weeks or three times a week for 10 weeks.

At the end of the treatment and for several weeks thereafter, the treated plants of both varieties did not differ from the untreated controls in plant height, number of tillers, ragged leaves, vein-swellings, nodal branches, panicles, and grains. Hence, neither of the antibiotic compounds suppressed the symptoms of the diseased plants.

DISCUSSION

Origin of the disease

The origin of the ragged stunt disease remains obscure although there are only three possibilities -- preexistence, mutation, and immigration. We suspect that the disease has long been present at a low level in rice fields. It may have been unnoticed because its symptoms are not obvious at certain rice growth stages, particularly when it appears together with rice grassy stunt, or when the disease is observed from a distance.

The disease is not limited only to the Philippines, Indonesia (Hibino et al. 1977; Palmer and Soepriaman 1977), and Thailand (Weerapat and Pongprasert 1978) because the occurrence of rice plants with similar disease symptoms in India and Sri Lanka has been verbally reported by several scientists. In almost all cases, the existence of the ragged stunt disease was first discovered by scientists with knowledge of symptoms of the disease. Hence, it appears that the disease was there.

A new rice virus disease

Besides rice ragged stunt, 14 rice virus and virus-like diseases are known in the world: black-streaked dwarf, dwarf, giallume, grassy stunt, hoja blanca, necrosis mosaic, orange leaf, stripe, transitory yellowing, tungro (including the tungro-like diseases), waika, yellow dwarf, yellow mottle, and yellow stunt (Ling 1972; Nishi et al. 1975; Plant Protection Institute 1974; Yokoyama et al. 1974). They differ from the ragged stunt disease but two of them, black-streaked dwarf and grassy stunt, have some unique similarities to ragged stunt disease.

Besides stunting, the black-streaked dwarf also causes rice plants to produce vein-swellings (Kuribayashi and Shinkai 1952; Shinkai 1966), but they are darker than those for ragged stunt. The vein-swellings of black-streaked dwarf that appear on culms can be grouped as tumors, which are more irregular in shape and wider and protrude much more than ragged stunt vein-swellings on culms. Also, rice plants infected by black-streaked dwarf produce more tillers and do not exhibit ragged leaves and nodal branches. Furthermore, they differ in species of insect vector. The black-streaked dwarf is transmitted by the small brown planthopper *Laodelphax striatellus* (Fallén), and has not been successfully transmitted by the brown planthopper *N. lugens* (Shinkai 1966), the vector of ragged stunt disease.

Although both grassy stunt and ragged stunt are transmitted by the same vector species *N. lugens*, in the same persistent manner, and without transovarial passage, they differ in symptoms such as degree of stunting, tillering, width of leaf blades, and appearance of ragged leaves, twisted leaves, vein-swellings, and nodal branches. Furthermore, they do not cross protect each other.

Consequently, ragged stunt is a new rice virus disease.

A member of acanthoviruses

The ragged stunt virus is a new member of plant reovirus group (Shikata et al. 1978). It appears to fall into the subgroup *acanthovirus*, a term proposed by Milne and Lovisolo (1977), who listed the following criteria for recognizing viruses of the subgroup:

1. Gramineae only are infected, with symptoms of darker green color (except pangola stunt virus), dwarfing, enation production, increased tillering, and suppression of flowering on at least some hosts.

2. Planthoppers (Delphacidae) propagatively transmit the viruses.

3. Plant or hopper material, homogenized in small amounts of saline, clarified by low-speed centrifugation, and injected into the vectors, should render them inoculative in 1-2 weeks. (Mycoplasmas would fail to be transmitted in this way.)

4. Thin sections of leaf enations and of infective hoppers reveal, in the cytoplasm, spherical virus particles about 70 nm in diameter containing dense cores of 50 nm diameter. Viroplasms appear in the light microscope as X-bodies. In plants, the viruses are phloem associated.

5. Simple dip or crush preparations of enations of swollen veins can be negatively stained for electron microscopy. Uranyl acetate reveals spherical particles of 65 nm diameter, with double capsids and also spiked cores about 50 nm in diameter. Phosphotungstate reveals smooth spherical cores about 55 nm in diameter, though very occasionally the outer capsid may be retained.

The available information on rice ragged stunt disease seems to fit most of the above criteria. Consequently, the ragged stunt virus may be a member of acanthoviruses.

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