Rice virus diseases

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CONTENTS

ILLUSTRATIONS

KEYS

TABLE

PREFACE

The rapid development of rice virology during the past few years and the increasing interest in virus diseases of the rice plant have led me to revise the pamphlet "Virus Diseases of the Rice Plant" (Ling. 1968a) published by The International Rice Research Institute.

The purpose of this publication is to present concisely the important information, available before 1971, pertaining to virus diseases of rice. It is also intended to help trainees, students, and farmers acquire fundamental knowledge about rice virus diseases, and to facilitate identification and control of the diseases. Therefore, the first portion of this publication covers general information about rice virus diseases and the second portion deals with specific diseases.

Much of the information available before 1967 in this publication can also be found in more detail in "The Virus Diseases of the Rice Plant" published by Johns Hopkins Press, Baltimore, Maryland, U.S.A.

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—K. C. Ling. *Los Baños, Laguna, Philippines. March 29, 1971.*

INTRODUCTION

Rice virus diseases in this publication refer to rice diseases caused by viruses or by presumptive mycoplasma. In other words, the diseases are caused by pathogenic agents which are infectious and multiply in host plants but do not belong to bacteria, fungi, parasitic phanerogams, or nematodes.

HISTORY OF VIRUS DISEASES OF PLANTS

Virus diseases were recorded as prevalent in certain cultivated crops over 300 years ago, however little was done to determine their causes until the middle of nineteenth century. Mayer (1886) investigated the mosaic disease of tobacco and found that the causal agent of the disease in juice extract was mechanically transmissible to healthy plants. Ivanowski (1892) demonstrated that the extract remained infectious after passing through a bacteria-retaining Chamberland filter. Later, Beijerinck (1898) concluded from his experiments with agar diffusion and serial inoculation that tobacco mosaic disease was not caused by microbes but by a "contagium vivum fluidum" (contagious living fluid) or a virus and that the causal agent reproduced itself in the living plant.

Since the term "virus" had been used in the Pasteurian sense as synonymous to bacteria, "filterable virus" was used for many years to designate infectious filter-passing entities. Nevertheless, "virus" alone has now become accepted because the old term gradually fell into disuse and perhaps the word "filterable" is not precise enough to specify the size of the filter pore that would alter the filterability. Although the study of viruses and virus diseases originated in

pathology, virology has now become a basic biological science in its own right and is an integral part of molecular biology.

DEFINITION OF VIRUS

Many definitions have been proposed for viruses. The changes in them, over the years, reflect the advancement of knowledge about viruses.

... [Viruses are] agents below or on the borderline of microscopic visibility which cause disturbance of the function of living cells and are regenerated in the process (Gardner, 1931).

... viruses appear to be ultramicroscopic size and obligate parasitism. They are the smallest units showing the reproductive property considered typical of life (Green, 1935).

Viruses [are] etiological agents of disease, typically of small size and capable of passing filters that retain bacteria, increasing only in the presence of living cells, giving rise to new strains by mutation, not arising *de novo* (Holmes, 1948).

... a virus [is] an obligately parasitic pathogen with dimensions of less than 200 millimicrons (Bawden, 1950).

The theoretical virus would be defined as a specific reproductive nucleoprotein or molecule which was, is, or may become infectious or pathogenic (Lwoff, 1953).

Viruses are submicroscopic entities capable of being introduced into specific living cells and reproducing inside such cells only (Luria, 1953).

... viruses [are] sub-microscopic, infective entities that multiply only intracellularly and are potentially pathogenic (Bawden, 1964).

... viruses should be separated from nonviruses by the use of a few discriminative characters: (a) Virions possess only one type of nucleic acid, either DNA or RNA. Other agents possess both types. (b) Virions are reproduced from their sole nucleic acid, whereas other agents are reproduced from the integrated sum of their constituents. (c) Virions are unable to grow and to undergo binary fission.... (d) Absence in the viruses of the genetic information for the synthesis of the Lipman system, the system responsible for the production of energy with high potential. (e) Viruses make use of the ribosomes of their host cells. This is defined as absolute parasitism (Lwoff and Tournier, 1966).

Viruses are now defined as follows: Particles made up of one or several molecules of DNA or RNA, and usually but not necessarily covered by protein, which are able to transmit their nucleic acid from one host cell to another and to use the host's enzyme apparatus to achieve their intracellular replication by superimposing their formation on that of the host cell; or occasionally, to integrate their genome in reversible manner in that of the host and thereby to become cryptic or to transform the character of the host cell (Fraenkel-Conrat, 1969).

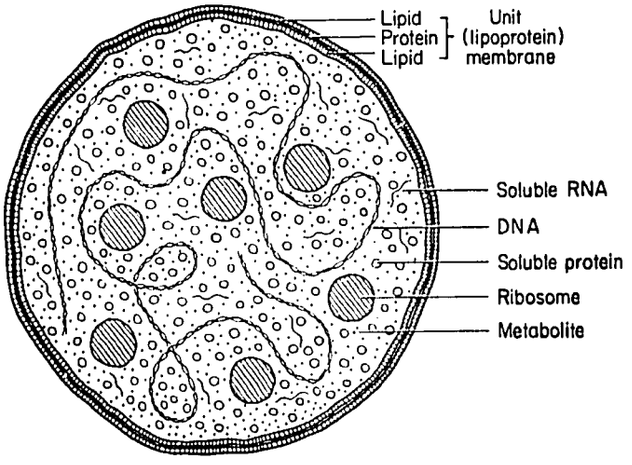
It is evident that a virus is submicroscopic, infectious (i.e., it can be transmitted by inoculation), multiplicable in living cells, a particle (not a cell), and it can produce new strains by mutation.

These properties, especially the first four, are essential in determining whether a plant disease is incited by a virus. Separating a virus disease from other causal organisms of plant disease such as fungi, bacteria, parasitic phanerogams, and nematodes can often be based on the sizes of their entities. Evidence of infectiousness is required to distinguish a virus disease from a physiological disorder. Evidence of reproduction is necessary to discriminate between a virus disease and a disorder induced by insect toxin. For instance, the leaf gall disease of rice, described by Agati and Calica (1949) in the Philippines, was later shown to be induced by toxins of *Cicadulina bipunctella* (Matsumura) and not by a virus transmitted by the insect because of the absence of multiplication of the agent in plants. Determining whether a disease is caused by a virus or by a mycoplasma (see below) can be based on whether the entity is a particle or not.

MYCOPLASMA

The study of "Mycoplasmology," a term first used by Klieneberger-Nobel (1967) during a conference in May 1966, was started by Nocard and Roux's paper "Le microbe de la peripneumonia" published together with their collaborators in 1898.

Mycoplasmas, previously known as pleuropneumonia-like organisms (PPLO), can be simply defined as polymorphic micro-



Schematic representation of a single cell of a mycoplasma (after Morowitz and Tourtellotte, 1962, and Ogata and Kushida, 1970).

organisms that are non-motile, that can be cultivated with difficulty on special media, and that lack a cell wall, being bound only by a unit membrane. The internal components are ribosomes and DNA strains.

One definition of mycoplasma was given by Edward (1967).

> Mycoplasmatales, excluding *M[ycoplasma] laidlawii*, may be defined as a group of microorganisms whose minimal viable units (elementary bodies) measure about 200 mμ. These cells enlarge during growth and have a potential for growing in branching filaments. Reproduction appears to take place by the development within the filaments, and possibly also in the cytoplasm of the larger cells, of further elementary bodies, and their subsequent release by fragmentation and disintegration of the filaments. The organisms lack a cell wall, and moreover are incapable of synthesizing cell wall components, such as muramic and diaminopimelic acids. Because of the lack of a cell wall, they are plastic and assume their characteristic pleomorphic morphology. Also associated with the lack of a cell wall are the properties of growing into a solid agar medium with the formation of characteristic colonies, comparatively easy lysis by osmotic shock, absolute resistance to inhibition by penicillin, and inhibition of growth by antibody. They require sterol (for example cholesterol) for growth, presumably to maintain the integrity and function of the cell membrane.

The definition does not include *M. laidlawii* merely because this species does not depend on cholesterol for growth.

The definition of mycoplasma given by Hayflick (1969) is as follows:

> Mycoplasmas, the smallest free-living microorganisms, are composed of minimal reproductive units as small as 125 mμ in diameter which enlarge to spherical or branching forms up to 500 mμ in diameter. The organisms lack a cell wall and are, in consequence of the effects of external physical forces, highly pleomorphic. They grow on lifeless media and the center of agar-grown colonies (10 to 600 μ in diameter) is often deeply embedded in the agar. Mycoplasmas are highly susceptible to lysis by osmotic shock, are resistant to penicillin, inhibited by specific antibody, and will tolerate low concentrations of thallium acetate. With the exception of *Mycoplasma laidlawii*, all require sterol for growth and multiplication. Despite a superficial resemblance, they differ from the L-phase of bacteria in that mycoplasmas do not derive from a bacterial parent—a fact that has been substantiated by immunological and nucleic acid homology studies. Futhermore, the bacterial L-phase is not dependent upon sterol and protein for growth and the minimal reproductive units are generally larger than those of the mycoplasmas.

The discovery of mycoplasma-like or PLT (psittacosis-lymphogranuloma-trachoma)-group-like organisms in plant tissues infected with so-called "viruses" such as mulberry dwarf, potato witches' broom, aster yellows, and paulownia witches' broom, was first reported by Doi et al. in 1967. At the same time, Ishiie et al. (1967) demonstrated that antibiotics of the tetracycline group could suppress the development of symptoms of mulberry dwarf

disease. Since then, plant pathologists have begun to realize that mycoplasma may also be a causal organism of plant disease and they have begun to doubt that all "virus" diseases are caused by viruses. Fulfillment of Koch's postulate for mycoplasmas as pathogenic organisms of plants was claimed by Lin and Lee (1969) and by Lin, Lee, and Chiu (1970) for sugarcane white leaf disease; by Hampton, Stevens, and Allen (1969) for alfalfa mosaic; and by Chen and Granados (1970) for corn stunt.

HISTORY OF VIRUS DISEASES OF RICE

Virus diseases of rice have been recognized since dwarf disease was first recorded in Japan in 1883. But mentek disease, which is suspected to be of viral origin (Hadiwidjaja, 1956; Ou, 1965) has been known in Indonesia since 1859. Nevertheless, before 1950, only three virus diseases—dwarf, stripe, and yellow dwarf—were described and characterized. In other words, before 1950, practically no report concerning virus diseases of rice existed anywhere in the world except in Japan and the Philippines.

The names of rice virus diseases are bewildering. Katsura (1936) started the confusion. Before he published his review paper in *Phytopathology*, rice dwarf was the only name of the disease in English although only a few papers about the disease had been published in English. Merely because of his preference, he used "stunt disease" in his paper. Since then, "dwarf disease," "stunt disease," "dwarf or stunt disease," "stunt or dwarf disease," and "dwarf (or stunt)" have appeared in the literature including textbooks. "Dwarf disease" has become the most common name in the last decade.

Before 1963, investigators of virus diseases of rice tended to emphasize the similarity of characteristics of a new disease to those of an already described one so that different diseases often received the same name. For instance, the virus diseases known as "stunt or dwarf" (Agati, Sison, and Abalos, 1941), "accep na pula" or stunt (Serrano, 1957), "dwarf" (Reyes, 1957), "dwarf or stunt" (Reyes, Legaspi, and Morales, 1959), and "tungro" or dwarf (Fajardo et al., 1962) are not identical to dwarf disease of rice described in Japan, but the investigators concluded the reverse.

After 1963, investigators tended to report a disease under a new name regardless of the appearance of the disease in the literature, resulting in several names for one disease. For instance, penyakit merah in Malaysia, yellow-orange leaf in Thailand, and leaf yellowing in India do not have any distinctly different characteristics from

tungro in the Philippines. Rice rosette seems to be identical to grassy stunt. Padi jantan in Malaysia does not differ from yellow dwarf in other countries. But the names are different.

The elimination of the confusion about the disease names is likely in near future because most investigators realize that identification of a rice virus disease should be based on all available information on physical and chemical properties of the virus, serological reaction, symptomatology, method of transmission, virus-vector interaction, species of vector, varietal reaction, etc. For instance, padi jantan was reported by Lim and Goh (1968) in Malaysia. Recently, due to the similarity of padi jantan to yellow dwarf, Lim (1970) proposed referring to padi jantan as yellow dwarf. Likewise the term tungro has appeared recently in Indian publications instead of leaf yellowing (John, 1968; Govindu, Harris, and Yaraguntaiah, 1968; Mukhopadhyay and Chowdhury, 1970; Raychoudhuri and John, 1970).

Before 1967, when the causal agent of a plant disease was unknown or obscure, it was often suspected to be a virus. As long as the experimental evidence indicated that the causal agent was infectious and submicroscopic, the nature of the causal agent was generally concluded to be a virus. This was true for rice virus diseases, too. Hence, the etiology of the disease was neglected because of limited facilities and techniques for studying the causal agent. When a specimen of diseased tissue was examined under an electron microscope, the investigator often searched only for virus-like particles, neglecting the rest, regardless of the pathogenicity of the particles.

About a decade ago, however, the first electron micrographs of a rice virus were shown by Fukushi et al. (1960) who not only determined the shape of particles of dwarf virus but also demonstrated the presence of dwarf virus in the diseased rice plants and in the virus-carrying insects, *Nephotettix cincticeps*. The infectious property was proved by the transmission of the virus after the insects were injected with the virus preparation (Fukushi and Kimura, 1959).

The association of mycoplasma-like bodies with rice yellow dwarf disease was first reported by Nasu et al. in 1967. Until now, mycoplasma has not been experimentally proved to be the causal organism of the disease, nevertheless a notion seems to prevail that mycoplasma may be the causal organisms of most diseases previously known as plant virus diseases of the yellows type. Therefore, the term "presumptive mycoplasma disease" has been used.

The causal agents of most rice virus diseases are either not clearly known or they lack confirmation. Similarly, it is difficult to

evaluate conflicting experimental results published by different investigators. Therefore, this publication emphasizes traditional concepts but it does not ignore new ideas or even contradictory results.

PARTICLES OF RICE VIRUSES

Particles of rice viruses are not all similar in shape or size. Rice viruses, arranged according to the shape and size (1nm [nanometer] = 10^{-9} meter = 1 millimicron) are:

A. Spherical or polyhedral shape

1. Dwarf—70 nm in diameter (Fukushi, Shikata, and Kimura, 1962).
2. Grassy stunt—70 nm in diameter (IRRI, 1966).
3. Black-streaked dwarf—60 nm in diameter (Kitagawa and Shikata, 1969b).
4. Yellow dwarf—55 nm in diameter (Takahashi, 1964).
5. Hoja blanca—42 nm in diameter (Herold, Trujillo, and Munz, 1968).
6. Yellow mottle—32 nm in diameter (Bakker, 1970).
7. Tungro—30 to 35 nm in diameter (Ou and Ling, 1967); 30 to 33 nm in diameter (Gálvez, 1968a). Yellow-orange leaf—30 nm in diameter (Saito et al., 1970).
8. Stripe—29 nm in average diameter (Saito, Inaba, and Takanashi, 1964); 25 to 35 nm, mostly 30 nm in diameter (Kitani and Kiso, 1968).
9. Orange leaf—15 nm in diameter (Ou and Ling, 1967).

B. Bacilliform or bullet-shaped

Transitory yellowing—120 to 140 x 96 nm (Chen and Shikata, 1968).

C. Rod-shaped

1. Rigid rod: Necrosis mosaic—275 or 550 nm in length, 13 to 14 nm in diameter (Inouye, 1968).
2. Flexible rod or flexuous thread: Hoja blanca—length variable, not determined, but diameter 8 to 10 nm (Shikata and Gálvez-E., 1969).

D. Mycoplasma-like organism

1. Yellow dwarf—in India (Sugiura et al., 1968), Japan (Nasu et al., 1967), Pakistan (Gálvez E. and Shikata, 1969), Philippines (Shikata et al., 1968), and Thailand (Saito et al., 1970). Padi jantan—in Malaysia (Singh, Saito, and Nasu, 1970).
2. Grassy stunt—mycoplasma-like bodies have been observed by Shikata (IRRI, 1968).

3. Giallume—in Italy (Belli, 1969; Pellegrini, Belli, and Gerola, 1969).

Most of rice viruses have not been thoroughly studied nor confirmed because of limitation of facilities and lack of bioassay techniques. Therefore, particles of some viruses have two shapes or sizes as reported by different groups of investigators. Yellow dwarf, grassy stunt, and giallume are now suspected to be caused by mycoplasma rather than viruses.

RICE VIRUS DISEASES AND THEIR DISTRIBUTION

Rice virus diseases reported in the literature can be grouped as follows (question mark means based on observation only):

A. Rice virus diseases for which transmission is well demonstrated:

1. Black-streaked dwarf (Japan).
2. Dwarf (Japan, Korea?).
3. Grassy stunt (Ceylon, India?, Malaysia?, Philippines, Thailand).
4. Hoja blanca (Western Hemisphere).
5. Necrosis mosaic (Japan).
6. Orange leaf (Ceylon, India?, Malaysia?, Philippines, Thailand).
7. Stripe (Japan, Korea, Taiwan?).
8. Transitory yellowing (Taiwan).
9. Tungro and tungro-like diseases:
   - Leaf yellowing (India).
   - Penyakit merah (Malaysia).
   - Tungro (India, Indonesia, Pakistan, Philippines, Thailand).
   - Yellow-orange leaf (Thailand).
10. Yellow dwarf and similar diseases:
    - Yellow dwarf (Ceylon, China?, India, Japan, Malaysia, Okinawa, Pakistan, Philippines, Taiwan, Thailand).
    - Padi jantan (Malaysia).
11. Yellow mottle (Kenya).

B. Possible rice virus diseases:

1. Giallume (Italy)—Belli (1969), Pellegrini, Belli, and Gerola (1969), and Baldacci et al. (1970) claim that the disease seems to be similar to rice yellow dwarf because of symptoms and the presence of mycoplasma-like bodies in the diseased tissues. However, the transmission has not been proved and the symptoms of the disease may not be identical with yellow dwarf.

2. Mosaic (Philippines)—The disease was observed and reported by Martinez et al. (1960). Although Martinez et al. transmitted the disease agent to maize seedlings by mechanical means, no information is available to indicate that the disease can be transmitted from rice to rice or from maize to rice. Until now, no other report on this disease has appeared, nor has anyone observed the disease in the field in the Philippines in recent years.

C. Diseases suspected to be of viral nature:

1. Bushy stunt (South Vietnam).
2. Chlorotic stunt (South Vietnam).
3. Chlorotic stunt with streaks (South Vietnam).
4. Mentek (Indonesia).
5. Yellow stripe chlorosis (India).
6. Yellow stunt (South Vietnam).

D. Virus diseases for which rice is a host plant:

1. Barley stripe mosaic virus.
2. Barley yellow dwarf virus.
3. Brome mosaic virus.
4. Maize dwarf mosaic virus.
5. Ryegrass mosaic virus.
6. Sugarcane mosaic virus.

E. Misidentified virus diseases of rice:

1. Leaf gall (Philippines)—The disease agent was claimed to be transmitted by *Cicadulina bipunctella* (Agati and Calica, 1949). It was actually due to insect toxin (Maramorosch et al., 1961).
2. "Stunt or dwarf" (Philippines) (Agati, Sison, and Abalos, 1941), "accep na pula" or stunt (Philippines) (Serrano, 1957), dwarf (Philippines) (Reyes, 1957), dwarf or stunt (Philippines) (Reyes, Legaspi, and Morales, 1959), and "tungro" or dwarf (Philippines?) (Fajardo et al., 1962)—Based on symptomatology, species of vector, and virus-vector interaction, these diseases are not identical to the dwarf described in Japan (Ling, 1969b; Ou and Ling, 1966; Ou and Rivera, 1969).
3. Rice rosette (Philippines) (Bergonia et al., 1966)—The disease is similar to grassy stunt (IRRI, 1964) based on symptomatology, species of vector, and virus-vector interaction.

Because mycoplasma-like bodies have been observed in tissues of infected plants under an electron microscope, yellow dwarf, grassy stunt, and giallume may be separated from the virus diseases and grouped as follows.

F. Presumptive mycoplasma diseases:

1. Yellow dwarf and similar diseases: Yellow dwarf (India, Japan, Pakistan, Philippines, Thailand) and padi jantan (Malaysia).
2. Grassy stunt (Philippines).
3. Giallume (Italy).

SYMPTOMS OF RICE VIRUS DISEASES

In all known virus diseases and presumptive mycoplasma diseases of rice, the causal agent often is present throughout the plant (systemic infection). The most commonly encountered symptoms are abnormal growth of the plant (teratological symptoms) and changes of color. The teratological symptoms are various degrees of stunting, increased or reduced number of tillers, twisting, crinkling or rolling of leaves, formation of galls on leaves and culms, and necrotic lesions on culms. In general, the changes of color on leaves of infected rice plants vary either from green to yellow to white or from green to yellow to orange.

Key for classifying rice virus diseases

A_1. Plants showing inconspicuous stunting, but reduced tillering
- B_1. Upright growth habit, premature death, orange-colored and rolled leaves.....ORANGE LEAF
- B_2. Spreading growth habit, oval to oblong faint chlorotic patches or fine faint mottling on leaves, brown necrotic lesions on basal parts of culms at later stages .....NECROSIS MOSAIC

A_2. Plants showing stunting and reduced tillering
- C_1. Leaves with chlorotic spots and white stripes
  - D_1. New leaves not unfolding properly but twisted and droopy..... STRIPE
  - D_2. New leaves unfolding normally.....HOJA BLANCA
- C_2. Leaves with mottling and yellowish streaks, crinkling of the first newly formed leaves when infected at an early stage of growth.....YELLOW MOTTLE
- C_3. Leaves with yellow or yellow-orange discoloration
  - E_1. Virus particles are bullet-shaped and persist in the vector.....TRANSITORY YELLOWING
  - E_2. Virus particles are spherical or polyhedral and do not persist in the vector.....TUNGRO (leaf yellowing, penyakit merah, and yellow-orange leaf)
  - E_3. Mycoplasma probably the causal organism, transmission unknown..... GIALLUME

A_3. Plants showing severe stunting and excessive tillering
- F_1. Galls on leaves and culms.....BLACK-STREAKED DWARF

F_1. No galls
  G_1. Leaves with chlorotic to whitish specks forming interrupted streaks.....DWARF
  G_2. Narrow, stiff, light-green leaves often with rusty spots .....GRASSY STUNT
  G_3. Leaves showing general chlorosis .....YELLOW DWARF (padi jantan)

TRANSMISSION OF RICE VIRUSES

Plant viruses are transmitted by mechanical means, insects, mites, nematodes, fungi, dodders, pollen, seed, grafting, budding, vegetative propagation, or soil. Among rice viruses, transmission through the seed has been extensively studied. Up to the present time, more than 36,000 seeds collected from infected rice plants have been tested by investigators in different parts of the world. No positive results have been obtained to demonstrate the transmission of any known rice virus through seeds. Rice viruses are only known to be transmitted by mechanical means, by insect vectors, or through soil. Most are transmitted either by leafhoppers or by planthoppers; no other methods have given reproducible results.

Only 11 species of insects are known to transmit rice viruses. Four of them transmit only one rice virus, others transmit two or more rice viruses. Only three rice viruses are transmitted by only one known species of insect and the rest are transmitted by two or more species of insects. All known species of insects that can transmit a virus can transmit the virus regardless of stages of growth of the insect (nymph or adult) or of sex.

Leafhopper-borne viruses of rice are very difficult to transmit by mechanical means. The reason, in addition to virus-vector-host interaction, may be the existence of an inoculation site in the plant tissue. Okuyama and Asuyuma (1959) injected the midribs of 31 rice seedlings with an extract of diseased leaves and obtained two seedlings that showed typical symptoms of rice stripe disease. Since then, others have not always been able to reproduce this result.

At the IRRI, we have inoculated rice seedlings with tungro virus from diseased leaves by the pin-prick method. Occasionally, a few seedlings become infected but the result is not consistently reproducible. We have not concluded that the tungro virus can be transmitted by mechanical means because the results could have been caused by contamination, i.e. a few insects might have inadvertently had access to the test plants in the greenhouse. On the other hand, our inconsistent results might have

occurred because occasionally we were able to introduce the inoculum to the inoculation site in the plant tissue by accident.

Furthermore, the vector of tungro virus, the rice green leafhopper *(Nephotettix impicticeps)*, is primarily a phloem feeder: more than 80 percent of their feeding tracks terminated at phloem tissue (Ling, 1968b). This leads to the suspicion that the inoculation site may be somewhere in the vascular bundle. Although the first-instar nymphs are very small, their feeding tracks also terminate at vascular bundles much more frequently than at any other tissues of the leaf. The positive transmission by the first-instar nymph therefore cannot rule out the possibility of the existence of an inoculation site. No available mechanical devise is able to introduce the inoculum to the site consistently so the positive transmission by mechanical means can only be obtained by chance.

Insect transmission terminology

Several terms used in reference to virus-vector interaction do not have uniform meanings in scientific literature. To make the terms precise they are defined below.

Acquisition feeding period: A time period that allows the insect to acquire virus naturally from a disease source. Authors often emphasize the shortest acquisition feeding period necessary to make the insect infective. Because it is difficult to determine exactly when a leafhopper starts to feed on diseased tissues, the acquisition feeding period generally refers to the time between confining the insect on a disease source and removing the insect from it.

Active transmitters: Insects that can transmit the disease after acquisition feeding. This term, expressed as a percentage, is used to show the proportion of a group of insects or of a colony of insects that are capable of transmitting the disease. At present, however, the percentage is a rough figure because investigators do not all use the same method of testing. The percentage may vary because of the age of insects, number of insects, length of acquisition feeding period, age of rice seedlings tested, variety of rice seedlings, source of virus, etc.

Consecutive transmission pattern: A pattern of transmission in which once an insect becomes infective, it transmits the disease continuously until it loses the infectivity and then it remains non-infective until death unless given an access to a disease source. The term generally refers to a daily time interval unless specified, for example, "hourly consecutive transmission pattern."

Incubation period in insect: The time between acquisition of virus and time when the insect becomes infective. Because the

exact moment when the insect starts to acquire the virus cannot be determined nor is it possible to determine the exact moment when the insect becomes infective during the inoculation feeding period, the term generally implies the maximum possible duration. That means that the incubation period is the time interval between the early possible moment of the acquisition feeding period and the last possible moment of the inoculation feeding period. Synonym: latent period in insect.

Incubation period in plant: The time between inoculation with a virus and the time when the inoculated plant shows the symptoms. Synonym: latent period in plant.

Infective insect: An insect that actually transmits the disease during the testing period. Antonym: noninfective insect.

Inoculation feeding period: The time it takes a viruliferous insect to inoculate a healthy plant upon feeding. The term generally refers to the time interval between introducing an insect to a plant and removing the insect from the plant. Investigators usually are concerned with the shortest inoculation feeding period that permits the plant to become infected.

Intermittent transmission pattern: The transmission pattern of an infective insect that fails to transmit the disease continuously during a given time interval. For example, a daily or hourly intermittent transmission pattern implies that the infective insect fails to transmit the disease every day or every hour before the insect completely loses its infectivity.

Nonpersistent: See p. 14. Antonym: persistent.

Number of disease-transmitting days: During a given length of time, the number of days during which an infective insect actually transmits the disease regardless of consecutive or intermittent transmission pattern of the insect. If an infective insect that has a consecutive transmission pattern is tested for a period longer than its retention period, the number of disease-transmitting days is theoretically equal to the retention period in days. However, the retention period is not applicable if the insect is given more than one acquisition feeding.

Persistent: See p. 14. Antonym: nonpersistent.

Retention period: The period in which an infective insect remains infective after an acquisition feeding. It generally means the longest retention period regardless of transmission pattern. This period is limited by the life span of the insect particularly if the insect carries a persistent virus. It is counted from the day when the insect acquires the virus to the last day that the insect is infective.

Transmissive ability: Ability of an insect or a group of insects to transmit a virus. It is determined by percentage of active trans-

mitters, length of retention period, number of disease-transmitting days, and other factors related to the efficiency of the insect to transmit the virus.

Transovarial (transovarian) passage: Transmission of a virus in the insect from one generation to the next through the egg. Synonym: congenital transmission or vertical transmission. The infective progeny are called congenitally infective insects.

Transstadial blockage: A term proposed here for the loss of infectivity of insects due to molting. Antonym: transstadial passage.

Transstadial passage: Retention of the virus by the insect even after molting. Antonym: transstadial blockage.

Viruliferous insect: The dictionary definition of viruliferous is containing or producing a virus. Therefore, "viruliferous insect" has been used quite imprecisely in the literature. The term refers to an insect that carries the virus, or that is capable of inducing a virus disease by feeding on a host, or that transmits the disease. Since the term "infective insect" covers part of the meaning and because only testing can prove that an insect is actually carrying the virus after being exposed to a diseased plant, "viruliferous insect" is defined as an insect that has been given access to a disease source. Antonym: virus-free insect, nonviruliferous insect.

Virus-free insect: An insect that neither has fed on a disease source nor, in species that have transovarial passage, is a progeny of an infective female. Synonym: nonviruliferous insect.

Persistent vs. nonpersistent.

The biological relationships between plant viruses and their insect vectors are not identical. Watson and Roberts (1939, 1940), therefore, developed the concept of grouping insect-borne viruses into persistent or nonpersistent based on their virus-vector interactions. Later, Sylvester (1956) proposed an intermediate group, semipersistent. There is steady progress toward clarification of these terms. Day and Venables (1961) have set up rather precise definitions for *persistent* and *nonpersistent:* A persistent virus: 1) has a long transmission time, 2) is recoverable from the haemolymph of a vector, 3) is transmitted following the molt of a vector, and 4) when purified and inoculated into haemocoele, makes the vector infective. A nonpersistent virus: 1) has a short transmission time, 2) is not recoverable from the haemolymph, 3) is not transmitted following a molt of the vector, and 4) when purified and inoculated into the haemocoele does not make the vector infective.

Kennedy, Day, and Eastop (1962) introduced new terms which

are less empirical and which give some indication of the location of the virus in the insect and the route followed by the virus. Instead of "nonpersistent" they suggested the term *stylet-borne*, "persistent viruses" become *circulative viruses*, and viruses that have a definite biological relationship (multiplication of virus in the vector) with their vectors are known as *propagative viruses*. One criterion of whether a virus is stylet-borne or circulative is transstadial passage.

Like other virus-vector interactions those between rice viruses and their insect vectors are not identical. The interaction of tungro virus and its vector is characterized by: 1) an absence of a demonstrable incubation period; 2) a gradual decrease of vector's infectivity; 3) a maximum retention period of less than a week; 4) transstadial blockage; 5) recovery of infectivity by reacquisition feeding; 6) increase in infectivity by prolonged acquisition feeding; and 7) a daily consecutive transmission pattern. Tungro virus is undoubtedly a nonpropagative virus in the vector (Ling, 1969a) since the virus does not persist in the vector. It seems to fit the term stylet-borne virus. But the insect does not transmit the virus in less than 1 or 2 minutes. In addition attempts to obtain the infected plants by mechanical means with reproducible results have been unsuccessful. These characteristics are different from those of typical stylet-borne viruses transmitted by aphids. Therefore, nonpersistent is the appropriate term for tungro virus (Ling, 1966).

Rice viruses that have transovarial passage must multiply in their vectors. If the virus did not multiply in the vector, the original virus in a single infective female would be so diluted in the progeny after a few generations that it could not cause infection. Thus this group of rice viruses could be called propagative viruses. But other rice viruses in the persistent group may not be just circulative.

For instance, Hsieh (1969) applied the microinjection technique to study the serial passage of transitory yellowing virus from insect to insect. He concluded that the virus multiplies in the vector but the virus is not transmitted through the egg. Hence, the term circulative should be avoided in rice viruses until the biological relationships of all leafhopper-borne rice viruses are clearly known. Furthermore, from a practical viewpoint transovarial passage is a more important phenomenon than the multiplication of the virus in the vector. Consequently, *persistent* may still be the appropriate term for rice viruses at least for the present.

Grouping of rice viruses by transmission

Based on the method of transmission and virus-vector interaction, rice virus diseases are grouped as follows:

Interaction of rice viruses and their vectors (Dash indicates information not available).

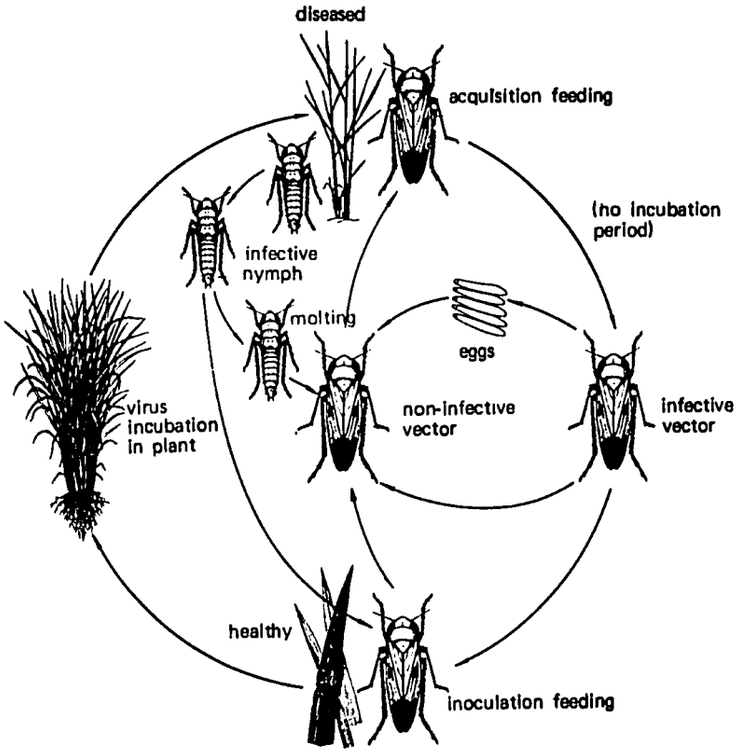
Disease [a]	Source of information	Incubation period in plant (days)	Vector	Active transmitters (%)	Shortest acquisition feeding period (min)	Incubation period in insect [b] (days)	Longest retention period (days)	Transstadial passage	Transovarial passage	Congenitally infective insects (%)	Persistent	Shortest inoculation feeding period (min)
Black-streaked dwarf	Japan	14-24	*L. striatellus*	32	30	4-35 (7-21)	58	yes	no	0	yes	5
			R. albifascia	50-73	15	7-25 (13)	49	yes	no	0	yes	15
			U. sapporonus	34	—	—	—	yes	no	0	yes	—
Dwarf	Japan	8-27	*N. apicalis*	23	—	—	—	yes	yes	—	yes	—
			N. cincticeps	0-69	1	4-58 (12-35)	65	yes	yes	32-100	yes	3
			R. dorsalis	2-43	30	9-42 (10-15)	93	yes	yes	0-64	yes	10
Grassy stunt	Philippines	10-19	*N. lugens*	3-50	30	5-28 (11)	40	yes	no	0	yes	5
Hoja blanca	Western Hemisphere	3-45	*S. cubanus*	—	—	—	—	yes	—	—	yes	—
			S. orizicola	5-15	15	5-37	14	yes	yes	60-94	yes	30
Orange leaf	Ceylon Philippines Thailand	13-21	*R. dorsalis*	7-14	5 hr	2-6	lifelong	—	—	—	yes	6 hr
Stripe	Japan	10-25	*L. striatellus*	14-54	3	5-21 (5-10)	47	yes	yes	42-100	yes	3
			R. albifascia	28-35	30	5-26 (12)	lifelong	yes	yes	—	yes	—
			U. sapporonus	—	—	—	—	yes	yes	—	yes	—

Transitory yellowing	Taiwan	10-11	*N. apicalis*	41-65	5	8-34 (9-16)	55	yes	no	0	yes	5-10
			N. cincticeps	35-71	15	21-34	—	yes	no	0	yes	—
			N. impicticeps	47	—	4-20 (10-12)	lifelong	yes	no	0	yes	—
Tungro	Philippines	6-9	*N. apicalis*	0-27	—	—	3	no	no	0	no	—
			N. impicticeps	83	30	2 hr, if any	5	no	no	0	no	7
			R. dorsalis	4-8	—	—	4	no	no	0	no	—
Tungro (leaf yellowing)	India	7-10	*N. impicticeps*	79	30	nil	2	no	no	0	no	15
Penyakit merah (tungro)	Malaysia	7-10	*N. impicticeps*	88	5-30	—	3-5	no	no	0	no	10-30
Yellow-orange leaf (tungro)	Thailand	6-15	*N. apicalis*	—	—	—	—	no	no	0	no	—
			N. impicticeps	82	20	—	6	no	no	0	no	15
			R. dorsalis	—	—	—	—	no	no	0	no	—
Yellow dwarf	Japan	27-90	*N. apicalis*	—	—	—	—	yes	no	0	yes	—
			N. cincticeps	88-96	10	26-40 (32)	103	yes	no	0	yes	5
			N. impicticeps	94	30	30-47 (34)	104	yes	no	0	yes	3
Yellow dwarf	Philippines	23-66	*N. apicalis*	69	—	20-35 (22-27)	38	yes	no	0	yes	—
			N. impicticeps	83	30	20-37 (20-26)	54	yes	no	0	yes	2-3
Yellow dwarf (padi jantan)	Malaysia	24-71	*N. impicticeps*	—	10	20-40	27	yes	no	0	yes	10
Yellow mottle	Kenya	7-20	*S. pusilla*	50	—	—	5	—	—	—	—	—

[a] Parentheses = other name.

[b] Parentheses = mostly or average.

Transmission cycle of nonpersistent rice viruses



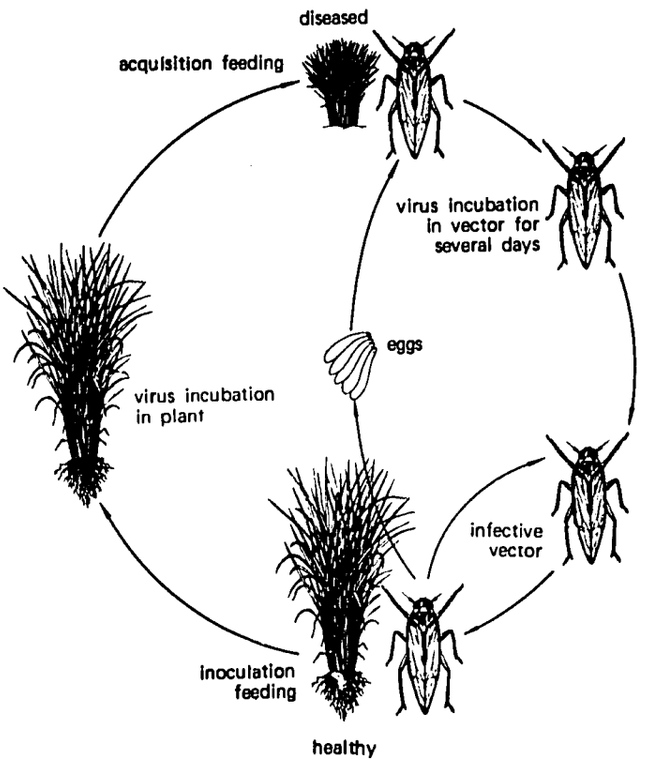
Insect → acquisition feeding → no demonstrable incubation period → infective insect → molting → noninfective insect → reacquisition feeding → reinfective insect → noninfective insect, the longest retention period is less than a week.

Infective female → eggs → noninfective progeny.

Nonpersistent viruses and their vectors

Tungro—*Nephotettix apicalis, N. impicticeps,* hybrids of *N. apicalis* and *N. impicticeps, Recilia dorsalis.*
Yellow-orange leaf—*N. apicalis, N. impicticeps, R. dorsalis.*
Penyakit merah—*N. impicticeps.*
Leaf yellowing—*N. impicticeps.*

Transmission cycle of persistent rice viruses without transovarial passage



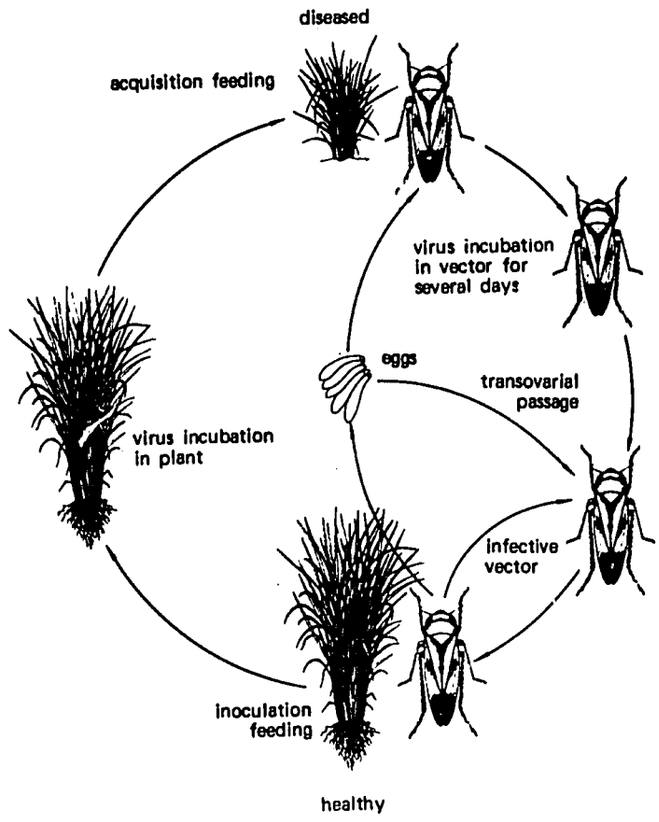
Insect → acquisition feeding → incubation period → infective insect → molting → infective insect, retaining infectivity almost for the rest of its life.

Infective female → eggs → noninfective progeny.

Persistent viruses without transovarial passage, and their vectors

Black-streaked dwarf—*Laodelphax striatellus, Ribautodelphax albifascia, Unkanodes sapporonus.*
Grassy stunt—*Nilaparvata lugens.*
Orange leaf—*Recilia dorsalis.*
Transitory yellowing—*Nephotettix apicalis, N. cincticeps, N. impicticeps.*
Yellow dwarf—*N. apicalis, N. cincticeps, N. impicticeps.*

Transmission cycle of persistent rice viruses with transovarial passage



Insect → acquisition feeding → incubation period → infective insect → molting → infective insect, retaining infectivity almost for the rest of its life.

Infective female → eggs → infective progeny.

Persistent viruses with transovarial passage, and their vectors

Dwarf—*Nephotettix apicalis, N. cincticeps, Recilia dorsalis.*
Hoja blanca—*Sogatodes orizicola.*
Stripe—*Laodelphax striatellus, Ribautodelphax albifascia, Unkanodes sapporonus.*

Key to rice viruses by transmission

A_1. Transmitted repeatedly by mechanical means
  B_1. Also transmitted by a beetle ..... YELLOW MOTTLE
  B_2. Also transmitted through soil.....NECROSIS MOSAIC
A_2. Transmitted by leafhoppers or planthoppers
  C_1. Virus does not persist in vector ..... TUNGRO (leaf yellowing, penyakit merah, and yellow-orange leaf)
  C_2. Virus persists in vector
    D_1. Transovarial passage absent.....BLACK-STREAKED DWARF, GRASSY STUNT, ORANGE LEAF, TRANSITORY YELLOWING, YELLOW DWARF (padi jantan)
    D_2. Transovarial passage present....DWARF, HOJA BLANCA, STRIPE
A_3. Transmission unknown ..... GIALLUME

Based on the interaction of virus and vector, the transmission cycles of leafhopper-borne viruses of rice are shown in the accompanying diagrams. Information about the virus-vector interaction of rice viruses is compiled on p. 16-20.

The major differences among the transmission cycles of rice viruses are that with nonpersistent viruses it is necessary to have diseased plant to complete the cycle, and the time duration required for a transmission from a diseased plant to a healthy plant by a virus-free insect is short (in hours) because of the absence of a demonstrable incubation period of the virus in the vector. In contrast with persistent viruses, once the insects become infective, the insects do not need to reacquire the virus to maintain their infectivity. But a long incubation period (in days) is definitely required for the insects to become able to transmit the disease after acquiring the virus. The difference between transovarial and nontransovarial transmission cycles is that in the former group, the insects retain their infectivity from one generation to the next without access to a disease source while insects in the latter group need to acquire the virus every generation to complete the transmission cycle.

INSECT VECTORS OF RICE VIRUSES

The insects that are known vectors of rice viruses and rice viruses transmitted by them:

Family Chrysomelidae

1. *Sesselia pusilla* (Gerstaecker) (beetle): yellow mottle.

Family Delphacidae

2. *Laodelphax striatellus* (Fallén): black-streaked dwarf and stripe.
3. *Nilaparvata lugens* (Stål) grassy stunt.

4. *Ribautodelphax albifascia* (Matsumura): black-streaked dwarf and stripe.
5. *Sogatodes cubanus* (Crawford): hoja blanca.
6. *Sogatodes orizicola* (Muir): hoja blanca.
7. *Unkanodes sapporonus* (Matsumura): black-streaked dwarf and stripe.

Family Cicadellidae

8. *Nephotettix apicalis* (Motschulsky): dwarf, transitory-yellowing, tungro, yellow dwarf, and yellow-orange leaf.
9. *Nephotettix cincticeps* (Uhler): dwarf, transitory yellowing, and yellow dwarf.
10. *Nephotettix impicticeps* Ishihara: leaf yellowing, padi jantan, penyakit merah, transitory yellowing, tungro, yellow dwarf, and yellow-orange leaf.
11. *Recilia dorsalis* (Motschulsky): dwarf, orange leaf, tungro, and yellow-orange leaf.

Some of these insects are shown in the accompanying sketches.

The following key, particularly under the family Delphacidae was prepared by Dr. T. Ishihara, Ehime University, Japan, for this publication.

Key to species of vectors of rice viruses

A_1. Mesothoracic wings (forewings) horny, veinless, mouth mandibulate ..... *Sesselia pusilla*

A_2. Mesothoracic wings leathery, containing veins; mouthparts forming a jointed beak, fitted for piercing and sucking

B_1. Ocelli on the frontal surface of head; middle coxae elongate, wide separate; hind coxae immovable; hind tibia with a conspicuous movable spur at apex; tegulae present.....Family DELPHACIDAE

C_1. Ovipositor slender, deeply curved dorsad in its basal half and straight or weakly recurved distally; the teeth on the upper margin numerous, even, and extremely small, and the basal end of the row not at all eleveted, so that the entire dorsal margin forms a single even curve parallel to the ventral margin; pronotal carinae usually straight; slender species ..... Genus *Sogatodes*

D_1. No spot on the clavus; style apex broad with the inner margins pointed and with a marked carina .....*S. orizicola*

D_2. A spot on the clavus; style apex small, slender and curved inward ..... *S. cubanus*

C_2. Ovipositor and pronotal carinae not as above

E_1. Hind basitarsus armed with one or several small spines; body and tegmina entirely brown or dark brown.....*Nilaparvata lugens*

E_2. Hind basitarsus not armed with such a spine or spines

F_1. Lateral carinae of pronotum convergingly divergent; male parameres short, about twice as long as the width ..... *Laodelphax striatellus*

F_2. Lateral carinae of pronotum divergingly divergent

G_1. Somewhat depressed species; male pygofer with a conspicuous ring-shaped base and parameres comparatively short, making an obtuse angle; tegmina in the brachypterous form (in the field only this form is known) marginally whitish .... *Ribautodelphax albifascia*

G_2. Slender species; male pygofer usual, with long parameres making an acute angle.....*Unkanodes sapporonus*

B_2. Ocelli on the dorsal surface of head; middle coxae short and close together, hind coxae movable; hind tibia without any spur; tegulae absent.....Family CICADELLIDAE

H_1. Connective linear, fused to aedeagus.....*Recilia dorsalis*

H_2. Connective Y-shaped, articulated with aedeagus ..... Genus *Nephotettix*

I_1. Aedeagus with elongated paraphyses and constricted below paraphyses; style curved; vertex with a submarginal black band.....*N. cincticeps*

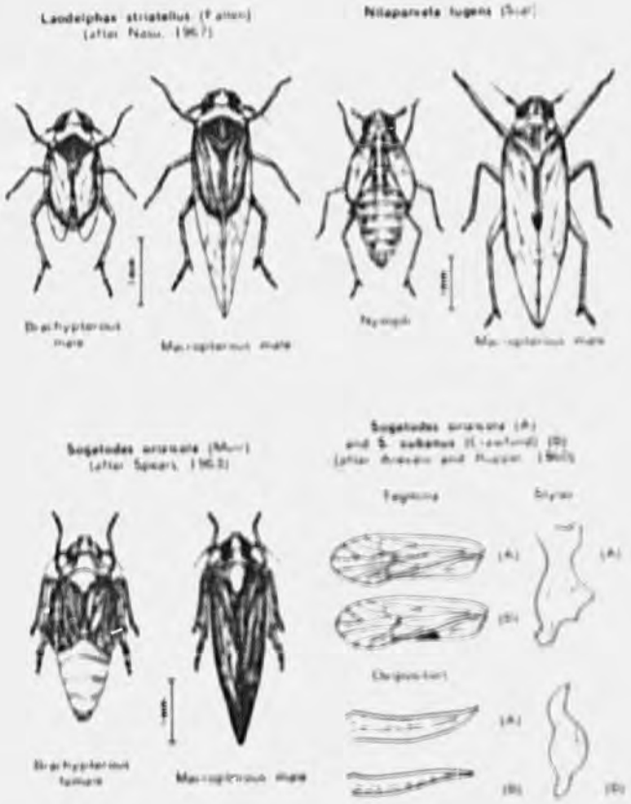
I_2. Aedeagus without elongated paraphyses and hardly constricted below paraphyses; style straight

J_1. Vertex with a submarginal black band; tegminal spots often present and confluent along the claval suture; aedeagus with a total of 10 to 23, mostly 14 to 17 teeth ..... *N. apicalis*

J_2. Vertex without a submarginal black band; tegminal spots present or absent, if present, not confluent along the claval suture; aedeagus with a total of 4 to 10, mostly 7 to 8 teeth ..... *N. impicticeps*

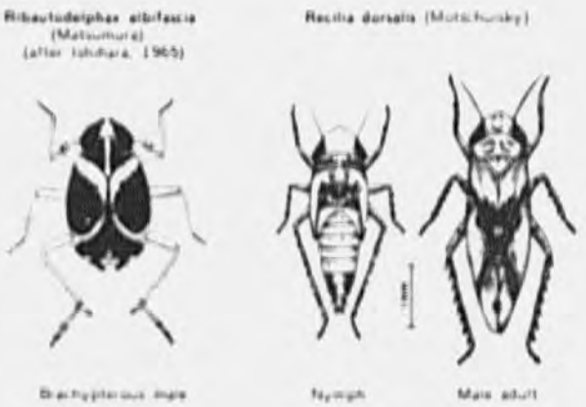
Laodelphax striatellus (Fallén)

Previously known as *Delphax striatella* Fallén in 1826, *Liburnia striatella*, *Delphax notula*, *Liburnia devastans*, *Liburnia nipponica*, *Liburnia minonensis*, *Liburnia giffuensis*, *Liburnia akashiensis*, *Liburnia maidoensis*, and *Delphacodes striatella*. Fennah (1963a) changed the genus name to *Laodelphax*, therefore the trivial name was *Laodelphax striatella*. However, Ishihara and Nasu (1966) changed it to *Laodelphax striatellus* because of the masculine gender of the name of genus *Delphax*.



Common name: small brown planthopper, smaller brown planthopper. The latter is commonly used by Japanese and the former seems to be appropriate

The morphology of this species was described by Lin (1967) and Nasu (1967): Male length, 3.5 mm (including tegmen), brachypterous female, 2 mm. General color yellow with black tinge; head pale yellow, vertex both laterals parallel, anterior end with two longitudinal black bands; compound eyes black, ocelli sometime deep red, antennae pale yellow with a thin longitudinal line on median; posterior of compound eyes dark brown; male scutellum mostly black or brownish black except apex, female scutellum mostly pale yellow tinged with gray; forewing pale yellow; apex somewhat brown, clavus end with dark brown spots particularly obvious in male; ventral surface of male body dark brown; male central thorax, pale yellowish brown; female central thorax, pale

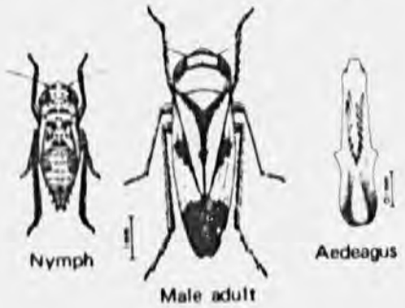


yellowish brown, mesothoracic laterals possessing black band, legs pale yellowish brown. Anal segment circular in shape, exterior side black, inner yellow having thick chitin on posterior portion with two spiny projections at center, anal style yellow. Male genitalia black, pygofer semicircular in shape, yellow near the end, style black, base wider, apex curved perpendicularly outward, possessing five toothlike projections at the end, aedeagus black, projected posteriorly, narrower at the end, female valvifer hook-like, lateral inner margin slanting.

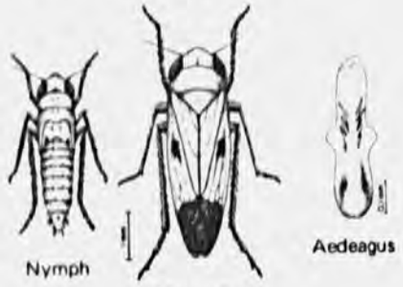
Nephotettix apicalis (Motschulsky)

Previously known as *Pediopsis apicalis* Motschulsky in 1859, *Pediopsis nigromaculatus*, *Thamnotettix nigropicta*, *Nephotettix*

Nephotettix apicalis (Motschulsky)



Nephotettix impicticeps Ishihara



apicalis in 1902, *Nephotettix nigropicta*, *Nephotettix bipunctatus* forma *apicalis*, and *Nephotettix apicalis apicalis*. In 1964, Ishihara revised the genus and named it again *Nephotettix apicalis*.

Common name: rice green leafhopper, green leafhopper, green rice leafhopper, black-streaked green rice leafhopper.

The morphology of this species was described by Nielson (1968): Medium size, slightly robust species. Length of male, 4.2 to 4.7 mm; female, 5.2 to 5.5 mm. General color green with black markings on elytra of males. Crown tannish brown or green, dark transverse line near anterior margin; pronotum tannish brown or green; elytra tannish brown or green with deep black markings along commissure, long irregular spot on corium next to middle of claval suture, deeply infuscated at apex; female with light-brown band on apex of elytra. Pygofer in lateral aspect about twice as long as wide,

caudal margin somewhat truncate; aedeagus in lateral aspect long, tubelike, broad on basal two-thirds, narrowed apically with notch at apex, several processes on dorsal margin at about middle of shaft, small spine on either side of lateral margin in ventral aspect; style in dorsal aspect with apices long and narrow, sides nearly parallel; female seventh sternum in ventral aspect with caudal margin notched medially.

Nephotettix cincticeps (Uhler)

Previously known as *Selenocephalus cincticeps* Uhler in 1896, *Nephotettix cincticeps* in 1902, *Nephotettix apicalis*, *Nephotettix bipunctatus cincticeps*, *Nephotettix apicalis* subsp. *cincticeps*, and *Nephotettix bipunctatus* forma *cincticeps*. Finally, Ishihara (1964) revised the genus and named it *Nephotettix cincticeps* again.

Common name: rice green leafhopper, green leafhopper, green rice leafhopper.

The morphology of this species was described by Nielson (1968): Medium size, rather robust species. Length of male 4.3 to 4.5 mm; female, 5.0 to 5.6 mm. General color green to gray with brown or black band on tip of elytra in male. Crown light green with distinct black transverse line near anterior margin; pronotum with anterior half green, posterior half brown; scutellum green; elytra light green broad brown or black band on apex in males; markings absent in females. Pygofer in lateral aspect about twice as long as wide, ventral margin with small tooth distally, caudal margin convex; aedeagus in lateral aspect long, somewhat tubelike, broad basally, slightly constricted subapically; expanded apically with lateral notch, dorsal margin with several processes, shaft with pair of distinct protuberances on middle and extending laterally in ventral aspect; gonopore subterminal on dorsal surface; female seventh sternum in ventral aspect with caudal margin truncate, slight protuberance medially.

This species has not been found in the Philippines although Ishihara included the Philippines under the distribution of this species in only one of his publications (Ishihara, 1965) and Baltazar (1969) listed this species in her checklist of Philippine plant pests. Baltazar's information however was originally from Ishihara, and Ishihara *(personal communication)* admitted he was mistaken.

Nephotettix impicticeps Ishihara

Previously known as *Cicada bipunctata* Fabricius in 1803, *Thamnotettix bipunctata*, *Nephotettix bipunctatus*, *Nephotettix apicalis*,

and *Nephotettix bipunctatus bipunctatus*. Ishihara (1964) changed it to *Nephotettix impicticeps* because the original name was preoccupied.

Common name: rice green leafhopper, green leafhopper, green rice leafhopper, Formosan green rice leafhopper, Taiwan green rice leafhopper, oriental green rice leafhopper.

The morphology of this species was described by Nielson (1968): Medium size, slightly robust species. Length of male, 4.3 to 4.5 mm; female, 4.9 to 5.5 mm. General color light yellowish green to green. Crown and pronotum light yellowish green, immaculate; elytra green with small brown or black spot at middle, brown or black band on apex in male, female unmarked. Pygofer in lateral aspect about twice as long as wide, ventral margin with small tooth at about middle, caudal margin convex; aedeagus lateral aspect nearly tubelike, dorsal surface with three or four narrow toothlike projections, each side of lateral margin with distinct rounded lobe in ventral aspect; style in dorsal with long narrow subtruncate apices; female seventh sternum in ventral aspect with caudal margin nearly truncate, slight indentation medially.

Nephotettix spp.

In addition to the above three species of *Nephotettix*, Ishihara and Kawase (1968) identified two new species in Malaysia, *N. malayanus* Ishihara et Kawase and *N. parvus* Ishihara et Kawase. These two species may occur outside Malaysia. In fact, *N. parvus* has been collected in the Philippines although the population was very low. These two species are likely to be vectors of rice viruses. Our preliminary studies indicated that *N. parvus* can transmit yellow dwarf. Ishihara and Kawase (1968) prepared two keys for differentiating five species of *Nephotettix*:

Key to Nephotettix *spp. by the crown and pronotum of the male insect*

A_1. Crown with the black submarginal band markedly present
  B_1. Anterior margin of pronotum without black tinge.
    C_1. Cephalic margin of head rounded.....*N. cincticeps*
    C_2. Cephalic margin projecting somewhat anteriorly .....*N. parvus*
  B_2. Anterior margin of pronotum tinged with black.....*N. apicalis*
A_2. Crown without the black submarginal band or with traces of it only behind the ocelli
  D_1. Crown a little longer medically than next to the eye, i.e., cephalic margin rather rounded.....*N. malayanus*
  D_2. Vertex markedly longer medially than next to the eye, i.e., cephalic margin projecting rather anteriorly about the middle .....*N. impicticeps*

Key to Nephotettix spp. *by the aedeagus.*

A_1. Aedeagus constricted about the middle
  B_1. A pair of paraphyses projecting perpendicularly to the aedeagus, i.e., the foremargins of both paraphyses making a line.....*N. cincticeps*
  B_2. A pair of paraphyses projecting obliquely.....*N. malayanus*
A_2. Aedeagus not constricted about the middle, stick-shaped
  C_1. A pair of paraphyses rudimentary, located near the middle portion of aedeagus.....*N. apicalis*
  C_2. A pair of paraphyses not rudimentary
    D_1. Foremargins of paraphyses greatly amplified.....*N. parvus*
    D_2. Foremargins of paraphyses not amplified.....*N. impicticeps*

Nilaparvata lugens (Stål)

Previously known as *Delphax lugens* Stål in 1854, *Liburnia sordescens, Delphax oryzae, Nilaparvata greeni, Kalpa aculeata, Delphax ordovix, Dicranotropis anderida, Delphax parysatis*, and *Hikona formosana*. Muir and Giffard (1924) transferred it to genus *Nilaparvata*.

Common name: brown planthopper, brown leafhopper. The former is commonly used.

The morphology of this species was described by Lin (1967) and Nasu (1967): Length 4.5 to 5 mm (including tegmen), brachypterous female 3 to 4 mm. General color dark brown or light brown; vertex lateral sides parallel, posterior cephalic margin slightly depressed; compound eyes black, ocelli dark brown, head ventral surface and antennae dark brown, front median line and lateral margins raised; pronotum and scutellum dark brown, having three longitudinal raised lines; forewing translucent, slightly brown tinged, veins yellowish brown, clavus hind margin central point with dark brown band; ventral surface of body and legs deep brown. Parameres of male genitalia simple with very shallowly furcate apex; male genital segment brown, pygofer and anal segment bulky portion with lighter coloration, anal style dark brown; pygofer somewhat elongated, cylinder shaped in ventral view, hind margin circular; style large, the end extending to half of anal segment; aedeagus base thicker, wavy shaped in lateral view, anal segment with two elongated downward projections; female first valvifer base with inner surface slightly oblong in shape, with middle part slightly curved.

Recilia dorsalis (Motschulsky)

Previously known as *Deltocephalus dorsalis* Motschulsky in 1859, *Thamnotettix sellata, Deltocephalus fulguralis, Thamnotettix storra-*

tus, Thamnotettix dorsalis, Togacephalus dorsalis, Sanctanus dorsalis, and *Inazuma dorsalis* (Ishihara, 1953). In 1968, Nielson suppressed *Inazuma* as a generic synonym of *Recilia* on the basis of the similarity of the male genitalia.

Common name: Zigzag leafhopper, zigzag-striped leafhopper, zig-zagged winged leafhopper, brown-banded rice leafhopper. The first one is commonly used.

The morphology of the insect was described by Nielson (1968): Small, linear species. Length of male 3.2 to 3.4 mm, female 3.7 to 3.8 mm. General color light gray. Crown and pronotum light gray with light infuscations of brown; elytra gray with conspicuous broad brown, zigzag longitudinal band. Pygofer in lateral aspect about 1.3 times as long as wide, caudal margin obliquely truncate; aedeagus in lateral aspect fused to connective; shaft tubelike, narrow, sharply attenulated apically, large sagittal groove on dorsal surface in dorsal aspect; style in dorsal aspect simple, apex narrowed; female seventh sternum in ventral aspect with caudal margin distinctly truncate.

Ribautodelphax albifascia (Matsumura)

The taxonomic position of the species has been settled only recently (T. Ishihara, *personal communication*). Previously this species was known as *Liburnia albifascia* in 1900, *Delphax albifascia, Delphacodes albifascia* (Ishii and Matsumoto, 1964), *"Delphacodes" albifascia* (Ishihara, 1965), *Delphacodes* (?) *albifascia* (Hirao, 1968a, 1968b), and *Ribautodelphax albifascia* (Shinkai, 1967; Ishihara, 1969).

The morphological characters of this species were described by Ishihara (1965, and *personal communication*): Somewhat depressed species; body (in brachypterous form) about 1.8 mm for male, 2.5 to 3.4 mm for female; body mostly black or dark brown, except vertex, median areas and longitudinal carinae of pronotum and of scutellum yellowish white. Antennae and legs light brownish. Tegmina black or dark brown, marginally with a whitish tinge except the costal margin. Vertex a little longer than the width. Pronotum clearly shorter than vertex. Scutellum large, more than twice as long as pronotum. In the laboratory only, the following macropterous form is known. The tegmina and wings both protruding the abdominal apex. Tegmina hyaline, slightly with brown tinge, infuscated at claval apices and without the conspicuous whitish fascia. Veins mostly brownish. Pronotum and scutellum mostly blackish and often without the pale markings along the median line in males, while they are present as in brachypterous form in females.

Sogatodes cubanus (Crawford)

Previously known as *Dicranotropis cubanus* Crawford in 1914 and as *Sogata cubana*. Fennah (1963b) transferred it to *Sogatodes*, which was first described by him.

Common name: rice delphacid.

The morphology of this species was described by Crawford (1914): Average length, 2 mm; width of vertex, 0.15; width of frons, 0.13; antennae I, 0.09, II, 0.18. General color brown, with whitish vitta on dorsum between lateral carinae; pronotum and scutellum darker outside of lateral carinae, sometimes almost black; lateral margin of pronotum whitish; vertex whitish; frons brown between carinae, latter white or pale; abdomen usually light brown; legs and antennae light brown; elytra hyaline, black at tip of clavus and with a brown crescent-shaped macula on apical margin. Body slender. Head short, narrower than prothorax, moderately produced before eyes; vertex about square, narrow; frons about twice as long as broad, or more, narrowed between eyes, sides nearly straight or slightly diverging; median carina forked at or slightly above ocelli; antennae reaching about to clypeus, I half as long as II, or less. Thorax rather slender; lateral pronotal carinae usually flexed outward or subobsolete before hind margin, sometimes percurrent to margin. Legs rather long; hind tibiae longer than femora; calcar long, large, thin, margin finely dentate. Elytra rather long. Male genitalia similar to *Megamelus approxima*, but genital styles about half as long, scarcely divergent.

The morphology of this species is similar to *S. orizicola*. The major differences: 1) *S. cubanus* usually is smaller than *S. orizicola*. 2) *S. cubanus* has a spot on the clavus between the common claval vein and the commissural margin. When the wings are folded, these spots form a saddle-shaped stigmata (Everett, 1969). 3) The ovipositor of *S. cubanus* is relatively narrower than that of *S. orizicola*. 4) The serrated edge of the ovipositor is more prominent in *S. orizicola* than in *S. cubanus*. 5) The apex of the style of *S. orizicola* is broad with the inner margins pointed and with a marked carina whereas the style apex of *S. cubanus* is small, slender, and curved inward (McMillian, 1963).

Sogatodes orizicola (Muir)

Previously known as *Sogata orizicola* Muir (Muir, 1926). Fennah (1963b) transferred it to *Sogatodes*. Confusion about the trivial name was introduced by Ishihara and Nasu (1966) who felt that *orizicola* was apparently intended to mean "habitant of the rice plant (*Oryza*)" and spelled erroneously. So they amended *orizicola*

to *oryzicola*. Many investigators followed their suggestion and used *oryzicola* instead of *orizicola*. Later, however, other biologists insisted that the original author can exercise the prerogative to give a name that departs from the strict rules for transliteration and name formation if he desires, so even Ishihara (1969) has used *S. orizicola* again.

Common name: rice delphacid, rice planthopper.

The morphology of this species was described by McGuire, McMillian, and Lamey (1960): Adult male about 3 to 4 mm long, fuscous in ground color, and with a light median stripe on the dorsum. The clypeus with two dark areas laterally leaving a light median stripe which widens towards the white vertex. The pronotum fuscous with two lateral dark spots and a mediodorsal white stripe. The mesonotum fuscous with lighter tegulae and a mediodorsal white stripe. The metanotum fuscous with a lighter triangle dorsally. The abdomen generally dark brown; the first abdominal tergum brownish yellow; all others dark with light margins and a very faint and narrow middorsal line. The anal segments black.

Legs very light testaceous, almost white. The forewings light testaceous with veins yellow except for r-m, apical part of R_4, M_{1-2}, M_3, M_4, Cu_{1-2}, and associated crossveins. Cells 4R usually darkened, but at time the basicostal area hyaline. Cells 2M, 3M, and 4M darkened as cells 2Cu, except for a small marginal area in the form of a crescent. The costa goes all the way around the wing, and the section of it on the vannal part white so that when the wings are folded over the abdomen, the two white costal veins cause the white dorsal stripe to extend almost the entire length of the insect.

The alate female light testaceous with the same white dorsal stripe. The abdominal tergites of the same general color as the rest of the insect except for a series of lateral marginal dark areas on each tergum which are homologous with the lateral dark stripes on the later nymphal stages. The wing light testaceous with a small darkened area at the point of anastomosing of the cubital and medial veins. The vannal portion of the costal vein white, as in the male.

The brachypterous female light testaceous throughout without any special markings. The wings reach only the hind margin of the third abdominal tergite, no clouding on the wings. The medial white stripe faintly present on the vertex and thorax.

The forms described are the light phase of two color phases found in this species. A darker phase in which the melanism is about twice that of the normal insect is also present.

Unkanodes sapporonus (Matsumura)

Previously known as *Unkana sapporona* Matsumura in 1935. Later, it was transferred to genus *Unkanodes* by Fennah (1956) and it was

named *Unkanodes sapporona*. The name was changed, however, to *Unkanodes sapporonus* in 1961 because the International Code of Zoological Nomenclature states that names ending in *-odes* are masculine. According to Ishihara (1969), it is the only known species in this genus.

The major taxonomic features of this genus were described by Fennah (1956): Body rather slender. Head little narrower than pronotum. Vertex longer than broad, its width as base not exceeding width of an eye, shallowly rounded at apical margin; carinae of vertex and frons distinct. Frons longer than broad, with median carina forked only at extreme base. Antennae cylindrical, basal segment two and a half times as long as broad, at least half as long as second. Length of pronotum and mesonotum combined equal to maximum width of latter. Pronotum tricarinate, lateral discal carinae almost straight; very weakly curved laterad, not reaching hind margin and not in line with mesonotal carinae. Mesonotum longer than head and pronotum together, tricarinate. Legs terete, not at all compressed, post-tibial calcar with about 22 teeth, basal segment of post-tarsus devoid of spines.

Ishihara (1966, and *personal communication*) described the morphology: Slender species. Body (including tegmina) about 4.5 mm for male, 4.7 mm for female, brachypterous form (including tegmina) 3 mm. Most light brownish, medially with a whitish tinge from vertex to scutellum. Antennae light brownish, slightly darkened at apex of the basal segment. Tegmina hyaline, broadly tinted with brown along the hind margin. Vertex slightly longer than the width. Pronotum about as long as head, with lateral carinae divergingly divergent towards the posterior margin and vanishing before reaching it. Scutellum large, longer than head and pronotum put together.

CONTROL OF RICE VIRUS DISEASES

The final results of studies of all diseases of plants must be their control or eradication and this is the all important problem for the grower. Methods of plant disease control may be divided into three major groups: prophylaxis, therapy, and immunization. Prophylaxis implies the protection of plants from exposure to the pathogen, from infection, or from the environmental factors favorable to disease development. Therapy refers to the cure of diseased plant by physical means or chemical compounds. Immunization concerns the improvement of resistance of the plant to infection and disease development. It is commonly known as disease resistance.

Prophylaxis is applied to a wide variety of control measures that may be subgrouped into exclusion, eradication, and protection. Exclusion includes measures designed to keep the pathogen from entering the sphere where the host is growing or to minimize the introduction of pathogen. Eradication consists of measures to eliminate the pathogen after it has become established in the sphere where the host is growing. Protection refers to measures used when it is assumed that the host will be exposed and that infection will occur unless the procedures are undertaken.

Before measures to control a virus disease can be formulated and applied it is often necessary to know the identity of the virus, methods of transmission, source of the virus, identity of the vector, source of the vector, activity of the vector, host susceptibility, etc. Control can best be achieved by using the knowledge of disease epidemiology to devise schemes to attack the virus or its vector at as many vulnerable points as possible.

Identification

Theoretically, the identification of a virus disease of a plant should be based on the characteristics of the causal agent that are discussed under the "Definition of virus," p. 2. Practically, the identification is often based on the symptoms of the disease. The major symptoms of all known rice virus diseases are listed in the key under "Symptoms of rice virus diseases," p. 10. But, it is often difficult to distinguish some rice virus diseases from physiological disorders in a field. The available knowledge on physiological disorders of the rice plant has been compiled by Tanaka and Yoshida (1970).

It is essential for identification of the disease to consider all existing phenomena, such as location of the field, distribution of diseased plants, and population of insect vector. The location of the field refers to the virus disease situation in the surrounding area. If the variety and plant age are identical in all fields, there should be no difficulty in finding diseased plants in adjacent fields if the disease is of a viral nature and transmitted by insects, unless the sources of seedlings are different and transmission occurs only in seedbed.

The distribution of diseased plants in a field is a clue to the nature of the disease. Plants that have a virus disease that is transmitted by insects are usually distributed irregularly in a field unless all rice plants in the field are infected. But for some rice virus disease, rice plants in the outer rows are more badly infected than those in the center of the field. That occurs when viruliferous insects migrate from the levee or the adjacent field. In contrast, in a field with a

nutritional disorder, plants in the outermost rows are often greener in color. The distribution of diseased plants in a hill serves a guide to the nature of the disease, if the individual hills originated from several seedlings. When a hill has both diseased and healthy plants, the disease is likely to be of viral nature because it is a result of systemic infection of individual plants.

Since most known rice viruses are transmitted by leafhoppers, the population of these insects, particularly at the time before the symptoms develop, is related to the incidence of virus diseases. Therefore, the nature of the disease can be traced by the relationship between the disease and the vector.

Symptoms of diseased plants are the best way to determine the nature of the disease. But the symptoms of several plants must be carefully examined and close attention should be paid to common characteristics of the diseased plants.

Some rice varieties that have unusual symptoms when infected can be used as indicators for disease identification. For instance, when infected with "S" strain of tungro virus, Acheh, FK 135, and Pacita have interveinal chlorosis which shows up as yellow stripes on the leaves. Shan-san-sa-san shows striping symptoms when infected with grassy stunt.

Periodic observation of the development of diseased plants can help identify the disease because all known rice virus diseases are systemic; the symptoms of the diseased plants usually do not disappear although plants sometimes recover. The systemic character of virus diseases can be used for identification in a practical way. For example, several diseased rice plants can be removed from field and transplanted to pots with soil different from that in the field and with sufficient fertilizer. If the symptoms disappear completely, it is unlikely that the disease is of a viral nature. If the symptoms remain until harvest, you can cautiously conclude that the disease is of viral nature, but you should be aware that causal factors other than virus may not have been completely removed from the treatment. An additional test is to ratoon the diseased plants. Because of systemic infection, the regenerated growth of virus-diseased plants often shows symptoms.

Since most rice viruses are transmitted by insect vectors, tests on the relationship between the disease and insects can provide evidence about the nature of the disease. Two principal types of tests can be made: keeping insects from healthy plants in the field or confining insects from the field on healthy seedlings. For instance, covering rice plants with a screen to prevent exposure of the plants to insects has been used to demonstrate the viral nature of the disease when the disease appears later only on uncovered plants (Ou and

Goh, 1966). Another approach is to confine a few insects collected from the diseased field on individual healthy rice seedlings for a short time as a method of inoculation. The transmission of the disease is established when the inoculated plants show symptoms. This test method was first used by a Japanese rice grower. Because the inoculation method involves insects, insect damage should not be overlooked. Insect infestation can retard plant growth and kill the plants. The degree of growth retardation however, depends upon the number of insects per seedling, number of insect feeding days, and susceptibility of the variety to the insect. The growth retardation caused by insect infestation often disappears in the later stages of plant growth when the insects have been removed. The rate of recovery is determined by the degree of insect damage. In contrast, growth retardation due to virus usually does not disappear.

Since rice viruses such as dwarf, tungro, and transitory yellowing can induce the starch accumulation in leaf blades, the reaction of leaf blade to iodine solution can serve as a confirmation. The test is made by staining the leaf blades with iodine solution after the chlorophyll has been removed by boiling the leaf in alcohol. The dark color formation indicates the presence of starch. A positive reaction is not substantial evidence about the nature of the disease however because no one really knows what factors other than virus can cause the accumulation of starch in the leaf blade.

Other methods such as examining inclusion bodies under microscope, examining purified virus particles under electron microscope, examining thin sections for viruses or mycoplasma-like bodies, and testing the serological reaction, have been used in laboratories. However, these methods require costly instruments and materials.

Prophylaxis

Most rice viruses are introduced to a field and disseminated in the field by insect vectors. The spread of insect-borne rice virus diseases, however, is determined by several factors such as source of the virus, population of the vector, movement of the vector, transmissive ability of the vector, and susceptibility of rice variety. Theoretically, any measure that eliminates, restricts, inhibits, or reduces the spread of the disease must be considered for its practical application in controlling the disease.

The source and perpetuation cycle of many viruses are a mystery to plant pathologists. Take leafhopper-borne rice viruses for example. Since the virus theoretically can only be perpetuated in the plant and in the insect, the source of the virus must be the

plant and the insect. But exactly which plants and insects are involved is unclear for many viruses. In general the sources of persistent viruses and viruses that have a wide host range are easier to discover than sources of nonpersistent viruses and viruses that have a limited host range. Under an overlapping cropping system, however, infected rice plants and volunteer plants from a previously infected crop are undoubtedly the source of the virus. Therefore reduction of the source becomes a problem of how to remove the infected plants and how to prevent the regenerated growth of infected plants after harvest.

Roguing is the common way of removing infected plants. Roguing, however, tends to be effective only when the spread of the virus is slow and mainly from infected to healthy plants within a crop. And, it is not successful unless only a few percent of the plants in the field are infected, unless it is done frequently and periodically, and unless the field and its surroundings are kept free from other susceptible hosts. Regenerate growth can be prevented either by plowing the stubble under the soil immediately after harvest or by draining water from the field and keeping the field dry enough to prevent any growth of plants.

Disease incidence is related to the presence of disease source in the field. For instance, during the last few years in the Philippines, the incidence of grassy stunt disease was high only on a few large farms such as the IRRI farm, Ledesma's farm (Bago, Negros Occidental), and Araneta farm (Valencia, Bukidnon). In other farmers' fields the incidence was very low or apparently no diseased plants were present. The difference was not due to the population of the vector, *Nilaparvata lugens*, nor was it due to differences in transmissive ability of the insects. "Hopper burn" which indicates a high population of the insects occurred not only in the large farms but also in small farmers' fields. Furthermore insects collected from small farmers' fields became as infective after acquisition feeding as the insects from the few large farms where grassy stunt was prevalent. The main difference was the presence of disease sources under overlapping cropping system used on the large farms.

Another example, occurred in Mindanao a fews years ago when tungro was prevalent in the Philippines. A field of Taichung Native 1, a variety highly susceptible to tungro, had no tungro disease although there was a high population of the vector, *Nephotettix impicticeps*, in the field. The reason was the absence of disease source in the surroundings.

These cases illustrate the importance of disease source in the spread of the disease. Consequently, reducing the disease source to a minimum is essential for limiting the spread of the disease.

In addition to infected plants, viruliferous insects are disease sources particularly when the virus persists in the vector. The source can be from outside of the field or within the crop because the insects spread the disease in two ways: by bringing the virus into a crop from outside and by spreading it from infected to healthy plants within the crop.

Van der Plank (1946) assumed that plants infected directly by incoming vectors will be randomly distributed whereas spread within the crop will produce groups of infected plants, and he devised a test for distinguishing between the two kinds of spread. If n consecutive plants are examined in sequence over a uniform area and of these x are diseased, then the number of random groups of two adjacent diseased plants ("doublets") d can be expressed $d = x(x-1)/n$. All larger groups of diseased plants are reduced to doublets. A doublet is any pair of adjacent diseased plants, a run three diseased plants being two doublets, a run of four, three doublets, and so on. The distribution belongs to the binomial series and to d is assigned a standard error of $\sqrt{d(1-p)}$, where $p = 2n$. For high values of n the distribution approaches the Poisson form, d having a standard error $\sqrt{d}$. When the observed value for d differs significantly from the calculated value, spread of the disease within the crop can be assumed. Otherwise, the distribution of diseased plants is at random. Hence, the spread is not from neighbor to neighbor.

For rice virus diseases, however, the number of doublets in the outermost rows of a field may not always be the indication of the spread of the disease within the crop. It is not uncommon for example to find plants with grassy stunt concentrated in the outer rows of a field so that a high number of doublets occurs in the outer rows. The reason is that the vector has two forms, macropterous (long winged) and brachypterous (with short or abbreviated wings). The latter form is unable to fly. When the viruliferous vectors migrate from levee into the field in the brachypterous form or in the nymphal stage, the plants in the outer rows are more likely to be inoculated and become infected. Consequently, doublets in the outer rows may not be the result of the spread of the disease within the crop but of incoming vectors. Or if plants have been infected in the seedbed before transplanting, the distribution of diseased plants may not reflect the spread of the disease.

The plant height of diseased plants of some rice virus diseases, such as tungro may indicate the time of infection and indirectly the source of vectors. The source of viruliferous insects is essential information for controlling the vector.

If a virus source is present, the incidence of insect-borne diseases mainly reflects the population and activity of the vectors. Because

of the variations among species of insect vectors and differences under various environmental conditions, it is difficult to generalize the population dynamics of the vectors. In general, however the leafhopper vectors need about a month to complete one generation (from egg to egg) in the tropics. If the growth duration of a rice variety is about 135 days, this is time enough for the insect vectors to pass through four to five generations if the insect starts living on the rice plant immediately after transplanting. Hence, due to the propagation of the insect, the insect population is generally higher later in the growth of the rice plant if climatic conditions remain constant.

The propagation rate of the insect is difficult to assess because of many variable factors. For instance, the number of eggs laid by a female *Nilaparvata lugens* can vary from 0 to 1,474 (Suenaga, 1963). However, in general, a female leafhopper can lay a few hundred eggs. If no insects die before laying eggs, one can calculate the number of progeny from a single female. For example, assuming a female insect lays 200 eggs, and 1:1 ratio of female and male in the progeny, after four generations, the progeny of a single female will be 200,000,000.

Control of insects, therefore, reduces not only the spread of virus diseases but also the direct insect damage to the rice plants. So, any effective measure or any efficient insecticide suggested by entomologists for the control of leafhoppers in rice field should be used until the population of the insects is reduced to a minimum although it is not usually possible to eliminate the insect entirely.

Virus-diseased plants may occur in the rice field even after the application of systemic insecticide. The infection may have taken place before the application of insecticide. Another possible cause relates to the shortest inoculation feeding period (see table of "Interaction of rice viruses and their vectors") which is generally a few minutes. If the time required by the insect to introduce the virus into the plant is shorter than the time the insecticide takes to kill the insect, the plant becomes infected. This has been proved by experiments in our greenhouse. However, since the insect is dead after feeding on the treated plant, it can no longer transmit the disease to the next healthy plant.

The time of application of insecticide for virus disease control is important. In all known cases (Lamey, Everett, and Brister, 1968; Lamey, Showers, and Everett, 1965; Ling, 1969d; Ling and Palomar, 1966; Palomar and Ling, 1966, 1968; Shinkai, 1962; Yasuo, 1969; Yasuo, Ishii, and Yamaguchi, 1965), other things being equal, the susceptibility of the rice plant to virus infection declines with older plants; older healthy plants are better able to tolerate infection than

young ones. Thus the percentage of diseased seedlings decreases when seedlings are inoculated at an older age. Plants inoculated at an older age may not show symptoms before harvest, but they may be infected because they sometimes show symptoms on the regenerate growth after ratooning. Similarly, the yield reduction is much greater when plants are infected at younger age. The yield often is practically not reduced when infection takes place at late stage of plant growth. Hence, steps to prevent the virus infection of rice plants must be focused on the early stages of plant growth.

Entomologists at IRRI made a field experiment with three rice varieties in 1966 when the tungro disease was prevalent at the IRRI farm. The yield of treated plots was significantly higher than that of untreated plot but there was no significant difference between the plot treated with insecticide up to 60 days after transplanting and the plot treated with insecticide continuously up to the harvest (*see* IRRI, 1967a). The best times to apply insecticide to reduce direct damage by insects may be different, however, because hopperburn, for example, often occurs at late stages of plant growth in the field.

The movement of viruliferous insects determines the rate of disease spread in the rice field. At present, how, why, and when the movement of rice insect vectors in the field occurs, is not well understood. Undoubtedly, the movement of the insects can be either active or passive. The distance that can be reached by adult insect or by planthopper of macropterous form is much greater than that of nymphs or of brachypterous forms of the same species. According to Miyashita et al. (1964), the mean distance of dispersal of *Laodelphax striatellus*, *Nephotettix cincticeps*, and *Recilia dorsalis* is 6 to 8 m, with a maximum of about 15 m in the nursery in 1 day. In the paddy field, the mean distance of the dispersal of *N. cincticeps* is about 13 m in 1 day and the maximum obtained was 41 m. On the other hand, Ishihara (1968) reported that a few specimens of *Nilaparvata lugens* were collected from a light on an ocean weather ship in the Pacific Ocean 500 km from the nearest land.

If the movement of the insects is stopped the disease cannot spread. It is easier to kill the insects, however, than to stop the movement of the insects. As mentioned before, the insect population is often higher at the late stage of rice plant growth. Because of high insect populations and because the availability of food is limited by the maturity of rice plant, the insects start to migrate at late stage of plant growth. Therefore, one idea is to apply insecticide to kill the insects during the period between panicle initiation stage and after the flowering stage of rice plant in fields where the population of insects and the incidence of virus disease are high. This

idea has two major flaws: Spraying at this time produces no economic return from the sprayed crop and it is difficult to spray all insects, especially those on the lower portion of the rice plants. Therefore, this practice has not become common.

Nevertheless, within one farm, the cost could be recovered from the adjacent fields. Moreover, when a field has a high population of insects, the insects are apparent on the stubble after harvest. Consequently, if a field has high incidence of virus disease before harvest and large number of insects on stubbles after harvest, there should be no difficulty in spraying insecticide to kill these insects to minimize the population of viruliferous insects in the farm and to reduce the virus source for adjacent fields.

Insect vectors are distributed widely in farms that have an overlapping cropping system. At the IRRI farm, we have collected infective vectors of grassy stunt disease in seedbeds, on volunteer rice plants, on weeds on the levees, and in idle areas as well as in paddies.

Hence, to control the vectors, the insects on volunteer rice plants and on weeds on levees should not be ignored.

Weed control on a farm having high incidence of rice virus disease, is needed because some weeds are known hosts of the virus and of insect vectors; other weeds can serve as temporary hosts for the insect vectors which means that the insect can survive on the weeds for some time, and also that the insect can lay eggs on the weeds and the eggs can hatch.

We have seen the seedlings showing typical tungro symptoms before transplanting, and we also obtained grassy stunt diseased plants from the seedlings of seedbeds transplanted to pots in greenhouse. Since the younger the seedling, the higher the susceptibility to virus infection, the seedbed should be well protected to keep the seedlings from becoming infected. If the seedlings show virus disease symptoms, about 2 weeks after transplanting it is better to replace them with healthy ones because seedlings infected at that stage will produce practically no grains.

The location of a seedbed in a virus-diseased area must be carefully selected. The seedbed should be away from virus-infected fields. Since the leafhopper vectors of rice viruses are phototropic (attracted to light) a seedbed should not be located in an area that is illuminated in the evening.

Seed or seedling treatment with insecticide often does not prevent the virus infection although the insect vectors die earlier on the treated seedlings than on the untreated control. Here again, the insect may have time to infect the plant before the chemical kills the insect.

Therapy

Physical means are not common in controlling viruses except for treating seed, seed pieces, or cuttings with heat. But there is no evidence to indicate that rice viruses are transmitted through seeds, so no studies have been made of heat treatment of rice seed for virus control.

Attempts made so far to free infected plants from a virus by the application of antiviral chemicals have been disappointing. The major difficulty is that to be effective the compound must inhibit virus infection and multiplication without damaging the plant. Virus multiplication is so intimately bound up with cell processes that any compound blocking virus synthesis is likely to have damaging effects on the plant. However, a few compounds such as 2-thiouracil and 8-azaquanine, used as foliar spray, as solution for watering plants, or for dipping plants, have been reported to prevent virus infection or to suppress symptom development and to diminish the virus concentration; but treated plants revert to the original condition after the treatment has ceased. None of these compounds has yet had any commercial application for controlling virus disease in the field nor have they been tried with rice plants infected with virus.

The use of antiviral chemicals against two rice diseases of the presumptive mycoplasma group, yellow dwarf and grassy stunt, has been tried. Application of tetracyclines has been reported to suppress symptoms of yellow dwarf (Sugiura, Kaida, and Osawa, 1969; Gálvez E. and Shikata, 1969; Singh, Saito, and Nasu, 1970; Sakurai and Morinaka, 1970). Applying four tetracyclines, tetracycline hydrochloride, chlortetracycline, dimethyl chlortetracycline, or oxytetracycline, as foliar spray or root dip to plants infected with grassy stunt caused no apparent differences in disease symptoms between treated and untreated plants. But a high percentage of plants died when treated with high concentrations of the compounds. Seedlings dipped in solutions of tetracycline hydrochloride, chlortetracycline, and dimethyl chlortetracycline, before or after inoculation of grassy stunt were no different from untreated seedlings in percentage infected (IRRI, 1968).

Resistance

The use of resistant varieties is one of the most effective, cheapest, and simplest ways to control crop diseases. This concept is applicable to rice virus diseases. There are four categories of resistance: 1) *immunity*, the plant does not become infected under any circum-

stances; 2) *resistance*, the plant processes external or internal factors which operate to reduce the chance and degree of infection; 3) *hypersensitivity*, the plant reacts by localized death of cells at the site of infection without further spread of virus; and 4) *tolerance*, the virus multiplies and spreads through the plant, but the disease produced is mild or negligible and yield loss is slight. The ideal is, of course, immunity, but it is rare. Resistance to infection and hypersensitivity are preferable. Tolerance is less satisfactory because it carries intrinsic dangers such as increasing both the virus sources and the chance of producing mutants.

There are three major steps in producing resistant varieties: 1) to develop a method of testing and screening and to standardize scales for measuring resistance; 2) to find a source of genes conferring resistance; and 3) to incorporate these genes with other desirable qualities in the crop. This section discusses only the first step.

The resistance of a rice plant to a virus disease can only be determined after the plant is exposed to the virus infection. The infection can be either natural or artificial. Therefore, varietal reaction to a virus disease transmitted by insect vector can be tested either by natural infection in the field or by artificial inoculation in the field or in the greenhouse. Both methods have advantages and disadvantages. The artificial inoculation method is generally preferable, however. This is the reason why varietal reaction to a rice virus disease is often tested by natural infection at first and then gradually replaced by artificial inoculation. Especially once the efficiency of the artificial inoculation method is improved, the natural infection method is often neglected.

For instance, varietal resistance to hoja blanca was first tested by natural infection in fields in Cuba and Venezuela (Atkins and Adair, 1957). Later, Lamey, Lindberg, and Brister (1964) perfected a mass screening technique by artificial inoculation for testing varietal reaction to hoja blanca in greenhouse at Louisiana State University, U.S.A. Similarly, Suzuki et al. (1960) started testing varietal resistance to stripe disease in the field by natural infection. Later, Sakurai, Ezuka, and Okamoto (1963) developed a "seedling test method" by artificial inoculation in Chugoku Agricultural Experiment Station, Japan. For tungro disease, Fajardo et al. (1964) made observations on varietal reaction in the field. In 1965, Ling (1967) developed a mass screening method by artificial inoculation at IRRI, the Philippines.

The workers on these three diseases can be considered as pioneers in the development of artificial inoculation methods for testing varietal resistance to rice virus diseases. Each group suc-

ceeded in conquering their technical problems. For instance, for tungro disease, the major problem, maintaining the infectivity of the insect, was eventually solved by daily reacquisition feeding.

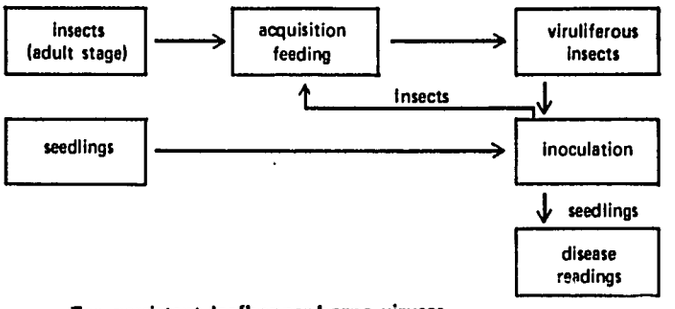
The varietal reaction to a virus disease by natural infection can only be tested in the field, therefore, it is often known as field test. Theoretically, this kind of test can be done even without knowing how the disease is transmitted. Kurosawa (1940) observed varietal differences to yellow dwarf in Taiwan in 1932. Hashioka (1952), also in Taiwan, studied varietal resistance to yellow dwarf by natural infection in 1946. These two can be considered the earliest records of varietal reactions to rice virus diseases. Actually, the transmission of yellow dwarf by *Nephotettix cincticeps* was suspected only in 1943 when it was described in the report of Kochi Agricultural Experiment Station (Shinkai, 1962). It was proved by Iida and Shinkai in 1950. In other words, Kurosawa and Hashioka did not know how the disease was transmitted when they made varietal resistance test by natural infection in the field.

The successfulness of a field test is determined by 1) occurrence of the disease, 2) incidence of the disease, and 3) uniformity of distribution of the disease in the test field. These factors are uncontrollable and vary according to location, year, population of insect vectors, etc. But, these factors can be checked by the results of replications of susceptible varieties in the test field. Viruliferous insects can be released into the field to improve the chances of disease occurrence, but the test may no longer be considered a natural infection.

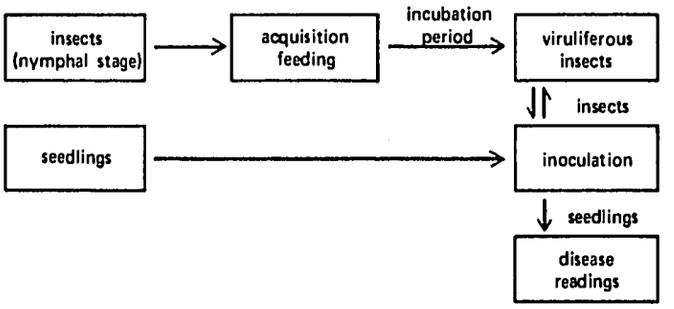
Varietal reaction to a rice virus disease can theoretically be tested by artificial inoculation in a field because after planting the field can be covered with a screen and viruliferous insects can be released under the screen to inoculate the seedlings. After inoculation, the insects can be killed with an insecticide and disease reading can later be taken in the field. So far, however, no one has tried because the successfulness of the test is determined by the size of the field. If the field is very small, it does not differ from the artificial inoculation in the greenhouse. If the field is large, many seedlings must be inoculated at one time, which leads to two major unsolved problems: how to prepare enough viruliferous insects, and how to distribute the insects evenly on every seedling.

The artificial inoculation method of testing varietal reaction to a rice virus disease transmitted by leafhoppers or planthoppers is shown in diagram (next page). Since the virus-vector interactions differ, methods for obtaining viruliferous insects differ, too. The methods, however, share several features: 1) the rearing and maintenance of a large constant supply of insects, 2) provision of a

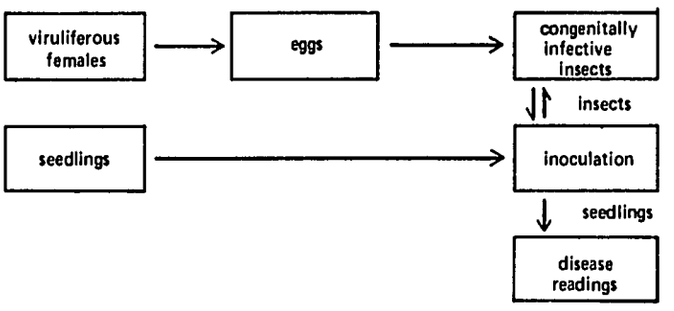
For non-persistent leafhopper-borne viruses:



For persistent leafhopper-borne viruses without transovarial passage:



For persistent leafhopper-borne viruses with transovarial passage:



ıgram of methods for testing varietal resistance to rice viruses.

nstant supply of healthy and diseased plants for the insects' ɛding material and for the insects' acquisition feeding, 3) prepara-ɔn of seedlings of rice varieties to be tested, 4) inoculation of the ɛdlings, and 5) the taking of readings of varietal reactions. Insect ctors of rice viruses can be reared on rice plants in cages. The cages ould have screens for ventilation and doors for moving plants and ɜects in and out. A constant supply of insects can be made by

maintaining a constant number of adult insects for oviposition in a cage, designated the "egg cage." The plants are kept in the egg cage for a fixed interval and then transferred to one of a series of cages. After the eggs are hatched, feeding materials are constantly supplied. Therefore, later, the insects, regardless of stage of growth, are available at a constant interval.

The method for obtaining viruliferous insects for inoculation depends on the virus-vector interaction. For nonpersistent leafhopper-borne viruses, expose the insects after the last molting to diseased plants for a few days and then use the insects for inoculation. Because the virus does not persist in the vector, the insects must be re-exposed to the diseased plants after every inoculation. Thus the viruliferous insects are used for inoculation once every day from morning to afternoon. Then from afternoon to the next morning, the insects are confined again on the diseased plants for reacquisition feeding.

Prepare viruliferous insects of persistent leafhopper-borne viruses without transovarial passage by exposing the insects to the diseased plants at the nymphal stage. Use the insects for inoculation after the incubation period of the virus in the vector is over They can be used repeatedly for inoculation until their death.

Viruliferous insects of persistent leafhopper-borne viruses with transovarial passage can be prepared the same way as those without transovarial passage. But the percentage of congenitally infective insects is always higher than the percentage of active transmitters (see the table of Interaction of rice viruses and their vectors p. 16), so infective females should be selected to establish a colony of congenitally infective insects that are used for inoculation.

The test seedlings can be prepared by soaking the seeds in water. When they germinate, transplant them in pots with soil mixed with adequate fertilizer (2.0 g $(NH_4)_2SO_4$, 0.8 g P_2O_5, and 0.8 g K_2O per kilogram of soil). At IRRI, seedlings we inoculate at the two- to three-leaf stage (11 to 13 days after soaking under Los Baños conditions). Of course, the age of seedlings at inoculation can be varied to fit the purpose of testing.

Inoculation can be made by confining the viruliferous insects in an inoculation cage. Moving the seedlings to the insects is easier than moving the insects to the seedlings. After the pots have been placed in the cage, disturb the insects to ensure that they are evenly distributed on each seedling. To prevent the migration of the insects towards the light cover the cage. After inoculation, shake or blow insects off the seedlings, remove the pots from the cage and keep the pots in the greenhouse for development of symptoms. The insects, however, can be kept in the cage for the next inoculation,

for reacquisition feeding, or to maintain the insect number in the egg cage.

How many seedlings to plant in each pot depends on the number of days required for symptom expression after inoculation. In other words, if the symptoms appear within a short period after inoculation, the number of seedlings in a pot can be increased; if the symptoms take a longer time to appear, the number of seedlings should be reduced. The principle is to provide enough space for the seedlings to grow until the symptoms develop.

The number of seedlings that can be inoculated per day is not determined by the number of seedlings in a pot, nor by the number of pots that can be accommodated in an inoculation cage, but by the number of available viruliferous insects because both the size of pots and size of an inoculation cage can be varied as desired.

The number of insects used for inoculating a seedling, designated n, is determined by: 1) percentage of active transmitters in an insect colony, designated a, and 2) the probability, P, that the seedling will be infected. By assuming that a seedling exposed to at least one infective insect will be infected, their relationship is described by the following equation (derived by Dr. Kwanchai A. Gomez, IRRI):

$$P = [1 - (1 - a)^n]$$

When three insects of a colony of 60 percent active transmitters are used to inoculate a seedling, the probability that the seedling will be infected is

$$\begin{aligned} P &= [1 - (1 - 0.60)^3] \\ &= [1 - 0.064] = 0.936 \end{aligned}$$

In other words, under the conditions given above, 93.6 percent of the seedlings in the cage are likely to be exposed to at least one infective insect.

The equation can be converted to calculate the number of insects required per seedling for inoculation:

$$n = \log(1 - P)/\log(1 - a)$$

When a colony of 30 percent active transmitters is used for inoculation and 96 percent infected seedlings is desired, the number of insects needed per seedling is

$$\begin{aligned} n &= \log(1 - 0.96)/\log(1 - 0.30) \\ &= \log 0.04/\log 0.70 = 9.02 \end{aligned}$$

That means that about nine insects are required for each seedling. Since the number of insects in the inoculation cage always de-

creases during handling and because of deaths, the numbers must be adjusted by using insects from a reserve cage where the extra viruliferous insects are confined.

More insects per seedling must be used to ensure a high percentage of infected seedlings after inoculation when a colony with a low percentage of active transmitters is used for inoculation. Attempts have therefore been made to increase the percentage of active transmitters by selection and cross breeding of infective females and males so that the number of insects used for inoculating a seedling can be reduced. Working with *Sogatodes orizicola*, McMillian, McGuire, and Lamey (1961) increased the active transmitters to 75 percent. Later, Hendrick et al. (1965) developed a colony containing 99 percent active transmitters. Gálvez (1968b) obtained a highly active colony of about 95 percent active transmitters. In *Nilaparvata lugens*, selection and cross breeding have been made to increase the active transmitters from about 30 percent to 54 percent (Ling and Aguiero, 1967), and later to 82 percent (IRRI, 1968). The percentage of increased active transmitters, however, often decreases gradually in successive generations to the original percentage when the selection of active parents is suspended and the insects are allowed to a free mating in the colony (Hendrick et al., 1965; Gálvez, 1968b; IRRI, 1968). Since it is laborious to select active parents continuously and it is difficult to get many progeny from a few selected parents, in the case of *N. lugens*, the remedy is to disturb the viruliferous insects in the inoculation cage once during the inoculation period to redistribute them on the seedlings which increases the chance of exposing all the seedlings to viruliferous insects (Ling, Aguiero, and Lee, 1970).

The reaction of seedlings to a disease can be noted after the symptoms express themselves or at a later stage of plant growth when the differences among varieties to the disease become obvious. Only two kinds of readings can be taken to indicate the reaction of a variety to a disease, however. They are percentage of infected seedlings and the severity of plant infection. The former indicates the resistance to infection, the latter, the tolerance to the disease. Therefore, the former is more useful. The latter should be used only when no varieties show consistently low percentage of infected seedlings.

Based on the percentage of infected seedlings, varieties can be divided into three groups: 1) *resistant*, indicating up to 30 percent infected seedlings, 2) *intermediate*, 31 to 60 percent infected seedlings, and 3) *susceptible*, 61 percent or more infected seedlings. This classification is, of course, arbitrary. The number of groups and the scales for each group can be changed but no matter what scales

are used or how many groups are classified, it is always an arbitrary classification system. For this reason, varieties in the same group may have significantly different percentages of infected seedlings.

The percentage of infected seedlings after inoculation is not determined entirely by the susceptibility of a variety; it may be influenced by such other factors as age of plant, infectivity of insects, escaping from inoculation, and number of seedlings inoculated. The percentage of infected seedlings of a variety may not be absolutely constant. Therefore, a variety placed in a resistant group in a preliminary test must be confirmed by further tests. Similarly, the number of seedlings for each variety should be increased to reduce the variation since the more seedlings of a variety tested, the higher the reliability of the result.

The best way to determine the severity of infection is to measure yield reduction. Practically, however, severity is estimated from the degree of growth retardation or the intensity of the symptoms because it saves time compared with waiting for the plant to mature and it saves space in the greenhouse that would otherwise be occupied by infected plants until maturity. The degree of growth retardation or intensity of symptoms of infected plants can simply be indicated by a group of numbers, abbreviations, or both. Or, a disease index can be calculated from the following equation:

$$\text{Disease index} = \frac{1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5}{n_1 + n_2 + n_3 + n_4 + n_5}$$

where 1, 2, 3, 4, and 5 represent, arbitrarily, five degrees of severity of infected plants and n_1, n_2, n_3, n_4, and n_5 represent numbers of observed seedlings in each degree of severity. The value of the disease index in the above equation cannot be greater than 5 or smaller than 1. However, the number of severity degrees and the representative number for each degree can be varied to suit individual cases.

The efficiency of the artificial inoculation method is determined by a steady supply of viruliferous insects, feeding materials, diseased plants, and seedlings for test. In 1 year, at IRRI, we test about 7,000 entries of rice varieties and lines, consisting of about 168,000 seedlings, for their reactions to tungro and grassy stunt. This requires about 3 man-years of work.

Seedlings tested by the artificial inoculation method are inoculated under somewhat forced conditions. Consequently, the results may not reflect the preference of the insect under natural conditions. In other words, a variety which is identified as susceptible by the test may have a low incidence of the disease in the field.

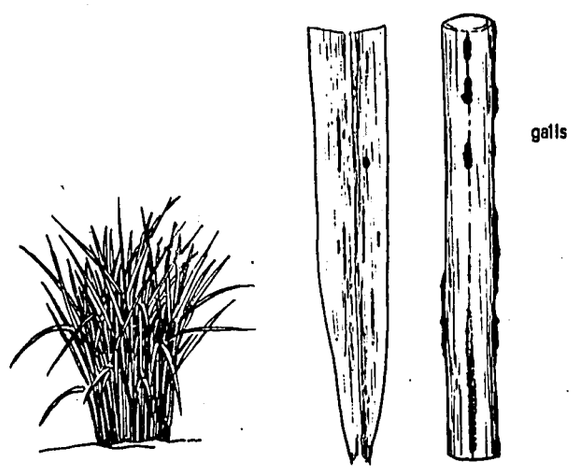
BLACK-STREAKED DWARF DISEASE

Black-streaked dwarf disease is translated from the Japanese name of the disease, kurosuji-ishuku-byo, given by Kuribayashi and Shinkai (1952). They were the first to report the transmission of the disease by the small brown planthopper and to point out the difference of the disease from dwarf and stripe. Black-streaked dwarf probably has been present in Japan for many years. The earliest recorded outbreak of the disease however, in the area of Nagano Prefecture, Japan was in 1941 (Kuribayashi and Shinkai, 1952).

The disease is only known to occur in Japan. According to Iida (1969), the disease has been found in six districts of Japan: Chugoku, Kanto, Kyushu, Shikoku, Tokai, and Tosan. It occurs in small patches of some fields and usually the yield losses are negligible. Rice plants inoculated before the 11-leaf stage, produce no grain however (Shinkai, 1962).

Symptoms

Phloem galls are characteristic of black-streaked dwarf. The galls appear as waxy, irregularly elongated protuberances extending along major veins on the lower surface of leaf blades, on the outer surface of leaf sheaths, and on culms. Galls develop as a result of hyperplasia of phloem parenchyma which increases the number of



Symptoms of black-streaked dwarf disease.

phloem parenchyma cells (Kashiwagi, 1966). The parenchymatous proliferations may erupt from the epidermis at several places forming a few gray or dark brown streaks of various lengths. The streaks usually appear on old leaves. Leaf blades, particularly the proximal parts, often become twisted (Iida, 1969). Plants that are infected survive until harvest although they are severely stunted. They have more tillers than normal unless infected at a very early stage of growth, dark-colored foliage, and no panicles or panicles which have emerged incompletely from the flag-leaf sheaths. The grains often have dark-brown blotches.

The plant age at the time of infection determines the degree of growth retardation. Inoculation at the three-leaf stage reduces plant height to about 80 percent of normal. The amount of reduction gradually decreases to nil as the plant age increases to 14-leaf stage at the time of inoculation (Shinkai, 1962).

A round inclusion body with a diameter of 6.5 microns is present in each of the proliferated cells of swelling tissue. But it is rarely found in the proliferated cells inside a large vascular bundle. The body may be an abnormal substance containing RNA (Kashiwagi, 1966).

Transmission

Black-streaked dwarf is known to be transmitted by three species of planthoppers: *Laodelphax striatellus* (Fallén) (Kuribayashi and Shinkai, 1952), *Unkanodes sapporonus* (Matsumura) (Shinkai, 1966), and *Ribautodelphax albifascia* (Matsumura) (Shinkai, 1967; Hirao, 1968a). *U. sapporonus* favors corn, wheat, and barley since rice is not its natural host. Transmission results of the disease by *Sogatella furcifera* or *Nilaparvata lugens* were negative (Shinkai, 1962).

The virus persists in the vectors. The proportion of active transmitters of *L. striatellus* is 32% (Shinkai, 1962), of *U. sapporonus*, 34% (Shinkai, 1966), and of *R. albifascia*, 50% (Hirao, 1968a) to 73% (Shinkai, 1967). The shortest acquisition feeding period is 30 minutes for *L. striatellus* (Shinkai, 1962) and 15 minutes for *R. albifascia* (Hirao, 1968a). The incubation period of the virus in *L. striatellus* is 4 to 35 days (Iida and Shinkai, 1969), but mostly 7 to 21 days (Shinkai, 1965). In *R. albifascia* the incubation period is 7 to 25 days with an average of 13 days (Hirao, 1968a). Most infective individuals remain infective until they become rather old. The longest retention period obtained is 58 days for *L. striatellus* (Shinkai, 1962) and 49 days for *R. albifascia* (Hirao, 1968a). Transstadial passage occurs. There is no evidence of transovarial passage.

The shortest inoculation feeding period is 5 minutes for *L. striatellus* (Shinkai, 1962) and 15 minutes for *R. albifascia* (Hirao, 1968a). The incubation period in the plant is 14 to 24 days (Shinkai, 1962).

The virus

The virus particles of black-streaked dwarf are spherical or polyhedral, 60 nm in diameter in purified preparations (Kitagawa and Shikata, 1969b). Their size is about 80 nm by center-to-center measurement within the crystalline inclusions that appear in infected cells of diseased plants and infective insects. By measurement of the longest axis for the polyhedral-shaped single particles *in situ* the diameter is 85 to 90 nm (Shikata, 1969).

The virus can be purified by the following procedure. The sap of diseased leaves is clarified with 30 percent carbon tetrachloride. It is then subject to differential centrifugation at 8,000 rpm (4,930 *g*) for 20 minutes and 20,000 rpm (30,800 *g*) for 60 minutes. The resultant pellet is suspended in 0.01M phosphate buffer at pH 7.0. After centrifuging at 8,000 rpm for 20 minutes, the supernatant fluid is subject to density-gradient centrifugation. The preparation is layered on the top of a column which contains, in order, 4 ml of 20 percent sucrose and 7 ml each of 30, 40, and 50 percent sucrose, and then centrifuged at 20,000 rpm for 60 minutes. A visible band 35 to 37 mm from the bottom of the tube is associated with high infectivity. The portion of the suspension forming the band is then removed from the column and centrifuged at 20,000 rpm for 60 minutes and after adding 30 to 35 ml 0.01M phosphate buffer at pH 7.0. The pellet is resuspended in the buffer solution and centrifuged at 8,000 rpm for 20 minutes to produce the virus preparation (Kitagawa and Shikata, 1969b).

The infectiveness of the preparation is determined by injecting the preparation into virus-free nymphs of *L. striatellus*. The infectiveness of the insects is determined by the seedling inoculation test. In the sap of diseased leaves the dilution end point is between 10^{-4} and 10^{-5} and in the extract of viruliferous insects between 10^{-5} and 10^{-6}. The thermal inactivation point of the virus in the sap of diseased leaves is between 50 and 60 C for 10 minutes. When the sap of diseased leaves and the extract of viruliferous insects are kept at 4 C, the virus remains infective for 6 days. Even when the diseased leaves are stored at −30 to −35 C for 232 days, the virus still remains highly infectious. The virus is stable in phosphate buffer solution at pH 6.98, in ammonium sulfate at pH 7.1, in tris buffer at pH 7.0, in distilled water, and in extracts adjusted to pH 6 to 9. The infectivity of the virus is lost when it is treated with chloroform or with mixture

of chloroform and *n*-butanol at a ratio of 1:1. It is not affected by the treatment with carbon tetrachloride, charcoal, deoxycholate, EDTA, and fluorocarbon (Difron S-3) (Kitagawa and Shikata, 1969a).

Host range

In addition to the rice plant, the 25 gramineous species are hosts of the black-streaked dwarf virus (Shinkai, 1962):

Alopecurus aequalis
A. japonicus
Avena sativa
Beckmannia syzigachne
Cynosurus cristatus
Digitaria adscendens
D. violascens
Echinochloa crusgalli
E. crusgalli var. *frumentacea*
E. crusgalli var. *oryzicola*
Eragrostis multicaulis
Glyceria acutiflora
Hordeum sativum var. *hexastichon*
H. sativum var. *vulgare*
Lolium multiflorum
L. perenne
Panicum miliaceum
Phleum pratense
Poa annua
Secale cereale
Setaria italica
S. viridis
Trisetum bifidum
Triticum aestivum
Zea mays

The virus also affects corn, wheat, and barley in the field, causing serious damage particularly to corn. Diseased plants are stunted, dark colored, and have galls (Obi, Kosuge, and Obi, 1960). Among the weeds, *A. aequalis* is found infected in the field most frequently (Iida, 1969).

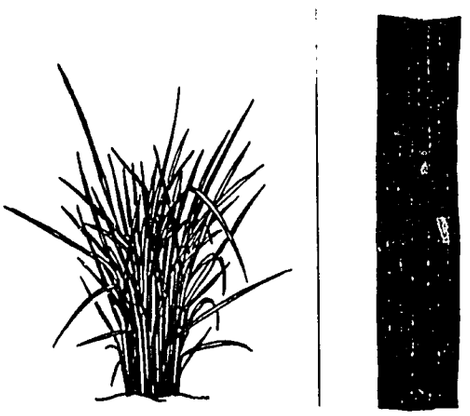
Varietal resistance

After observing varietal reaction to black-streaked dwarf in the field during an outbreak of the disease in the Kanto district, Ishii, Takahashi, and Ono (1966) reported that Gangsale Bhata and Maratelli were resistant. By testing the reaction of 43 varieties to the disease by seedling inoculation before transplanting in the field, Morinaka and Sakurai (1967) found that Amareriyo, Bluebonnet, Hatadavi, Loktjan, Modan, Pusur, Tadukan, Tetep, and Tsao-ta-tsu were resistant. Morinaka and Sakurai (1968) developed a test method. Viruliferous insects are prepared by exposing diseased plants to the insects at the first or second instar for 3 days. Three to four weeks later the insects are used for inoculating the seedlings. Thirty seedlings of each variety are exposed to about 50 insects for 2 days and then transplanted to a seedling box. Four weeks after transplanting, the percentage of diseased plants, degree of stunting, and vein enation are determined. Morinaka and Sakurai (1968)

tested 382 varieties. They consider the following varieties to be resistant:

Bandang putih
Benong 130
Bhasamanik
Chiem Chank
Hu-nan-tsao
Jaguary (gl.) A
Koentoelan
Mao-tzu-tou-sien-tao
Nomai
Pa-shih-tze-sien
Philippine No. 4
Ta-yeh-tze
Tetep

Based on the reactions of progenies of Tetep x Chusei-shin-senbon and Yamabiko x Tetep, the resistance of Tetep to the black-streaked dwarf is controlled by one major dominant gene. Modifying genes may be present that exert some influence on the degree of resistance (Morinaka, Toriyama, and Sakurai, 1969).



Symptoms of dwarf disease.

DWARF DISEASE

Dwarf disease is a translation of Japanese "ishuku-byo" which means a disease of dwarfing or stunting. That is the reason for the occurrence of "rice dwarf" and "rice stunt" in English literature. However, nowadays, *rice dwarf* is more commonly used by investigators to refer to the disease.

Rice dwarf is the best-known virus disease in the world although the distribution of the disease seems to be limited to Japan and Korea. It is not only the first virus disease of rice identified but the study of it contributed to classical knowledge of plant virology. For instance, it was the first plant virus disease found to be transmitted by an insect, it provided the first evidence for the multiplication of a plant virus in an insect, and it was the first virus detected within both hosts, the infected plant and viruliferous insect, by an electron microscope.

The disease seems to have been in Japan for a long time. According to Katsura (1936) and Fukushi (1969), the historic outbreak of the disease occurred in 1897. Previously, minor outbreaks had been known locally, since the disease was first discovered in Shiga Prefecture in 1883. The relation of leafhoppers to the disease was first experimentally demonstrated by a rice grower, Hatsuzo Hashimoto. He planted young rice plants in a glass container and enclosed them in a cheesecloth cage, introducing numerous leafhoppers. Consequently, he discovered the causal relation of leafhopper to

rice dwarf in 1894. But, he did not report his tests, so the leafhopper species with which he worked is not known.

The first report on the etiology of the disease was given by K. Takata in 1895 and 1896, when he traced the cause of the disease to the insect, "mon-yokobai" *(Recilia dorsalis)*. The Shiga Agricultural Experiment Station undertook studies on this disease and published results of experiments with insect pests in 1898 to 1908. In 1900, it pointed out that "tsumaguro-yokobai" *(Nephotettix cincticeps)* was the true cause of the disease and several other species of insects had no connection with the disease. It is evident that the disease was at that time entirely attributed to the leafhopper. In 1901, N. Takami reported that rice dwarf was due to leafhopper, tsumaguro-yokobai, a claim that he later withdrew.

In 1910, H. Ando reported a study in which leafhoppers captured in the vicinity of Tokyo in 1905 were reared on dwarf-diseased rice plants. The progeny of the second or third generation produced infections on healthy rice plants and noninfective leafhoppers became infective after feeding on diseased plants for about 15 days. Thus, he concluded that rice dwarf was not caused by the leafhopper but by an unknown causal agent carried by the leafhopper. Fukushi (1969) however, pointed out that the work of N. Onuki with leafhoppers was earlier than Ando's. In 1899, Onuki found that the leafhoppers, *N. cincticeps*, captured in the vicinity of Tokyo were unable to produce rice dwarf in healthy plants. But through the experiments conducted at Ando's suggestion in 1902, he showed that these leafhoppers became infective if they had fed on diseased plants. Thus, it became evident that the leafhopper was the carrier of the causal agent, the nature of which was unknown.

This finding was confirmed by the Shiga Agricultural Experiment Station in 1908. Consequently, the true role of the leafhopper as a vector of rice dwarf was established by N. Onuki at the Imperial Agricultural Experiment Station (predecessor of National Institute of Agricultural Sciences) and T. Nishizawa at the Shiga Agricultural Experiment Station. Later, Kunkel (1926) pointed out that the rice dwarf disease was the first virus disease of plants shown to be transmitted by an insect.

The disease occurs in the following districts of Japan: Chugoku, Kanto, Kinki, Kyushu, Shikoku, Tokai, and Tosan but not in Hokkaido or most parts of Hokuriku and Tohoku (Iida, 1969), although most basic studies on rice dwarf disease were made in Sapporo, Hokkaido. The disease has also been observed in Korea (Park, 1966).

The yield loss caused by the disease is determined by the plant

age at the time of infection. Plants inoculated earlier than the 11-leaf stage produce practically no grain. When inoculated at the 11-leaf stage, the yield reduction is about 80 percent; at the 12-leaf stage, 20 percent; at the 13-leaf stage, 6 percent; at the 14-leaf stage or later, insignificant (Shinkai, 1962). Similar results were obtained by Ishii, Yasuo, and Yamaguchi (1970).

Symptons

Fukushi (1934) described the symptoms of rice dwarf as follows. The first visible symptom of the disease manifests itself as yellowish white specks along the veins of newly unfolded leaves. These specks, which develop before the leaves unfold, are yellowish-green to yellow when viewed by diffused light. By holding the leaf up to the light the specks become distinct, being yellowish white to white. The specks elongate and spread out along the leaf parallel to the midrib, forming fine interrupted streaks. These range from mere dots to an area several millimeters in length and from 0.2 to 1 mm in width. The succeeding leaves invariably show the white specks, while the lower, previously formed leaves exhibit no signs of the disease. On the leaf which shows the first visible symptoms of the disease the specks may be confined to the lower part of the leaf blade or to only one side of the midrib near the base of the leaf. On the succeeding leaves conspicuous specks develop in abundance and connect with each other, forming almost continuous streaks along the veins.

Although it is not unusual to find the disease in the seedbed, it usually appears in late June when the rice plants have been transplanted to paddy field. The symptoms of the disease become most pronounced at about the middle of July in Japan.

Following infection the growth of the rice plant is highly arrested. The diseased plant becomes remarkably stunted, the internodes are shortened, and numerous diminutive tillers develop producing a rosette appearance. Affected plants tend to develop a dark green foliage. The root growth becomes inhibited with only small roots that extend horizontally. Plants infected at the early growth stages often remain alive until harvest but produce no panicles or a few worthless ones.

The amount that plant height is reduced by the disease is determined by the plant age at the time of infection. The younger the plant is at the time of inoculation, the higher the reduction in plant height. The height reduction decreases from 70 percent to nil when plant age increases from the three-leaf to the 14-leaf stage at the time of inoculation (Shinkai, 1962).

The leaf blades of the diseased plant show accumulation of starch when tested. The accumulation is thought to be due to the slowness of starch translocation (Daikubara, 1904).

Studies of sections of diseased leaves show chlorotic modifications in the mesophyll cells adjacent to some vascular bundles. In sections mounted in water, the chlorotic tissues are lighter in color or nearly colorless. The chloroplasts in these cells are light colored and smaller in size and number. In cells where the chloroplasts have disintegrated, intracellular inclusions are usually present. The bodies are round to oval or irregular, measuring 3 to 10 by 2.5 to 8.0 microns. Thus they are considerably larger than the host nuclei (2.5 to 3.5 microns in diameter) near which they are situated. They contain many vacuoles of various sizes. Though more common in the mesophyll, one or two inclusion bodies are also sometimes found in the epidermis (Fukushi, 1931). Hirai et al. (1964) also found large spherical inclusions which stained violet with Giemsa. In the leaf sheath of the diseased plant, the inclusions are in parenchyma cells surrounding vascular bundles. Similar inclusions are also in adjacent cells that lack chloroplasts. In the leaf blade, these inclusions are in clusters in chloroplast-deficient parenchyma cells, probably corresponding to the location of visible, white-streak lesions.

Transmission

Rice dwarf is known to be transmitted by three species of leafhoppers: *Recilia dorsalis* (Motschulsky), first reported by Takata in 1895 and 1896 (Iida, 1969), confirmed by Fukushi (1937); *Nephotettix cincticeps* (Uhler), appeared first in the report of Shiga Agricultural Experiment Station in 1900 (Iida, 1969); and *N. apicalis* (Motschulsky) (Nasu, 1963). In most areas *N. cincticeps* plays the major role in transmission of the disease.

Fukushi (1934) was unable to transmit the disease through the seeds produced on infected plants, or by growing seedlings in soil so that the roots came in close contact with those of infected plants. A series of sap transmission experiments all gave negative results. Inoculation with macerated tissues of viruliferous insects likewise brought about no infection. Shinkai (1962) did not succeed in getting transmission by *N. impicticeps* Ishihara.

The virus multiplies in the insect vectors as shown by evidence of transovarial passage (Fukushi, 1933), by serial transfers of the virus from insect to insect by injection (Kimura, 1962a), and by the presence of the clusters of the virus particles in insect tissues (Fukushi et al., 1960) and mycetomes (Nasu, 1965). The percentage of active transmitters varies widely among the insect colonies collected from

different localities. The proportion of active transmitters is about 23 percent for *N. apicalis* (Nasu, 1963), 0 to 69 percent for *N. cincticeps* (Shinkai, 1962), and 2 to 43 percent for *R. dorsalis* (Hashioka, 1964). The shortest acquisition feeding period is 1 minute when nymphs of *N. cincticeps* at the first to the second instar are tested, and 30 minutes for *R. dorsalis* (Shinkai, 1962). The incubation period of the virus in *N. cincticeps* is 4 to 58 days, mostly 12 to 35 days (Iida and Shinkai, 1969). In *R. dorsalis*, it is 9 to 42, days, mostly 10 to 15 days (Shinkai, 1962).

Most infective insects retain their infectivity for life, but they may not transmit the disease every day. The longest retention period obtained is 65 days for *N. cincticeps*, and 93 days for *R. dorsalis* (Shinkai, 1962). The insects retain infectivity after molting. The virus is congenitally transmitted to the offspring from infective females but not from infective males. Thirty-two to one hundred percent of the offspring of an infective *N. cincticeps* female are congenitally infective; 0 to 64 percent are for *R. dorsalis* (Shinkai, 1965). From 1 to 38 days (with an average of about 15 days) must elapse before most of the nymphs from infective *N. cincticeps* become infective although a few individuals may transmit the disease immediately after they emerge from eggs. Most of these nymphs retain their infectivity during all the nymphal stages and for as long as 88 days through their adult life, without renewed access to a source of virus.

Considerable variation occurs in the infectivity of different leafhoppers. Some of them infect plants consistently on consecutive days while others do so only at great intervals. The virus can be passed from a single infective female through eggs to six succeeding generations. And there is no evidence of a progressive decrease either in the percentage of infective insects or in their infectivity (Fukushi, 1969). For *R. dorsalis*, the percentage of congenitally infective insects decreases remarkably as the insect generations pass. The insects of the fourth generation of an infective female often are not infective. The nymphs of an infective female begin to transmit the disease 3 to 14 days after hatching (Shinkai, 1962, 1965). The shortest inoculation feeding period is 3 minutes for *N. cincticeps* and 10 minutes for *R. dorsalis* (Shinkai, 1962).

Rice plants remain susceptible from the one-leaf to the 13-leaf stage (the 16th leaf is the last). The incubation period in the plant is 8 to 10 days until the 10-leaf stage of infection. Subsequently, the period lengthens with advancing leaf stage. If infection occurs at the 13-leaf stage, the incubation period is 27 days (Shinkai, 1962).

The virus has a deleterious effect on its vectors. The average life span of infective females of *N. cincticeps* is 12.1 ± 3.7 days and of noninfective ones. 16.6 ± 2.1 days. The mean fecundity for infective

females is 26.1 ± 41.0; of noninfective ones, 73.7 ± 17.4. The survival rate of nymphs is 31.1 percent for offspring of the infective female and 46.2 percent for the offspring of noninfective female (Nakasuji and Kiritani, 1970). Similarly, the offspring of infective *R. dorsalis* die earlier (Shinkai, 1962).

The virus

The first electron micrographs of the rice dwarf virus was made by Fukushi et al. (1960). The virus particle is an icosahedron about 70 nm in diameter, the diameter of long axis is about 75 nm and short axis about 66 nm. Each particle has 32 capsomeres which are composed of five or six tubular structural units, and 180 structural units in all on its surface. The capsomeres on the surface of the virus particles are separately projected from the capsids. The hollow tubes are about 6 nm in diameter and 9.5 nm in length (Kimura and Shikata, 1968).

The purified virus contains 11 percent RNA. The base composition is quanine 21.8%, adenine 28.4%, cytosine 21.6%, and uracil 28.2%. The mole ratios of adenine to uracil and quanine to cytosine are both close to unity. The RNA is double stranded (Miura, Kimura, and Suzuki, 1966). The helix-to-helix distance in this double-helix structure is 1.3 nm along the common axis of the helices (Sato et al., 1966). The double helical model for the structure of rice dwarf virus RNA is also supported by results obtained from optical rotatory dispersion and circular dichroism (Samejima et al., 1968).

The virus can be purified by the following procedure. Fifty grams of diseased leaves are cut into small pieces and ground with the addition of 200 to 500 ml of M/30 phosphate buffer, pH 6.8. Then the sap is expressed through cheesecloth and centrifuged at 6,500 *g* for 30 minutes. The top layer is stirred with 10 or 20 percent volume of chloroform for 5 minutes and centrifuged at 1,600 *g* for 20 minutes. The supernatant fluid is centrifuged at 26,000 *g* for 60 minutes. After removing the supernatant fluid, the pellet is suspended in M/40 tris buffer pH 7.2, and centrifuged at 1,600 *g* for 20 minutes. The supernatant fluid consists of a high concentration of virus particles when examined under an electron microscope (Toyoda, Kimura, and Suzuki, 1965). This method can also be used for purification of the virus from viruliferous insects (Fukushi, Shikata, and Kimura, 1962).

For further purification, for instance to remove the enveloping materials of the virus particles, the virus preparation is treated with phospholipase of snake venom or pancreation. After the treatment,

the virus is eluted from a DEAE-cellulose column with 0.2 to 0.25 M NaCl (Toyoda et al., 1965). Further purification can also be made by density-gradient centrifugation (Kimura, Kodama, and Suzuki, 1968).

The infectivity of the virus preparation is tested by injecting a small amount (1/3000 ml) of the preparation into virus-free *N. cincticeps*. The infectivity of the injected leafhoppers is determined by the infection of seedlings after they are inoculated by the insects (Fukushi and Kimura, 1959). The incubation period in the injected insects is 8 or 12 days to 33 or 37 days at 18 C (Kimura, 1962a). The dilution end point is between 10^{-3} and 10^{-4} for the sap of the diseased leaves, between 10^{-4} and 10^{-5} for the extract of the eggs from infective females. The thermal inactivation point is between 40 and 45 C for 10 minutes. The virus *in vitro* remains infectious at 0 to 4 C for 48 hours but not 72 hours. When the viruliferous insects and the diseased leaves are frozen and stored at −30 to −35 C, the infectivity is maintained up to a year (Fukushi and Kimura, 1959; Kimura and Fukushi, 1960).

The virus concentration in the infected rice plant reaches its maximum 40 days after inoculation. At this time, sap from the leaves and stems is infectious at a dilution of 10^{-3} and that from roots at a dilution of 10^{-2}. The sap from the yellow-green portions of the diseased leaves is infectious at a dilution of 10^{-4}; the sap from the green portions is less infectious (Kimura, 1962a).

When rabbits are injected intramuscularly with an emulsion of the partially purified virus in Freund's adjuvant, a high titer antiserum is obtained (Kimura, 1962b).

Host range

In addition to the rice plant, the following plant species are hosts of the rice dwarf virus (Shinkai, 1962; Iida, 1969):

Alopecurus aequalis
A. japonicus
Avena sativa
Echinochloa crusgalli var. *frumentosa*
E. crusgalli var. *oryzicola*
Glyceria acutiflora
Hordeum sativum var. *hexastichon*
H. sativum var. *vulgare*
Oryza cubensis
Panicum miliaceum
Paspalum thunbergii
Phleum pratensis
Poa annua
Secale cereale
Triticum aestivum

Varietal resistance

Based on field observations and field tests, the following varieties were resistant to dwarf disease: Dahrial, Gangsale Bhata, Hyaku-

nichi-to, Kaladumai, Kaluheenatii, Kuan-yin-sien, Loktjan, Pe Bi Hun, and Peh-rih-tao.

Based on the artificial inoculation tests (Kimura et al., 1969; Ishii, Yasuo, and Yamaguchi, 1969), the following varieties were resistant: Bluebonnet, C203-1, Chiem Chank, Dahrial, Depi, Intan, Kaeu N 525, Kaladumai, Karalath, Loktjan, Peta, Tadukan, and Tetep (Sakurai, 1969; Ishii, Yasuo, and Yamaguchi, 1969).

Based on the number of insects (under natural conditions), preference of the insect, hatching rate, mortality of nymphs, body weight, and uptake of plant sap, Dahrial, Kaladumai, Tadukan, and Tetep were also resistant to the vector, *N. cincticeps* (Ishii, Yasuo, and Yamaguchi, 1969).

GIALLUME DISEASE

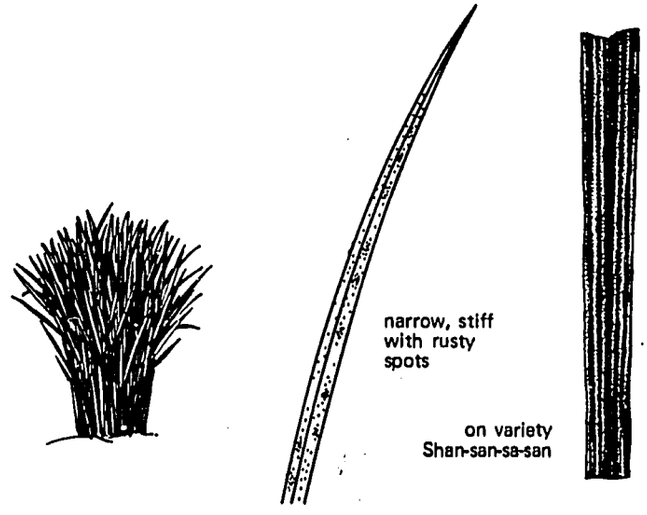
Giallume is an Italian expression for yellowing. The disease has been observed in Italy since 1955 and it has become more common in recent years (Baldacci et al., 1970). The diseased plants are stunted, the number of tillers is slightly reduced, and the leaves are yellowed. The way the disease is transmitted is not known. However, mycoplasma-like bodies have consistently been found not only in ultrathin sections of the diseased leaves but also in clarified extracts of diseased plants. The bodies are polymorphic, 120 to 420 nm in size, bounded by a unit membrane, and they contain many ribosomes (Pellegrini, Belli, and Gerola, 1969). Belli (1969), however, described the bodies in clarified extracts as highly polymorphic for intermediate and large forms, spherical for small forms, 60 to 800 nm, and bounded by a unit membrane. The investigators (Belli, 1969; Pellegrini et al., 1969; Baldacci et al., 1970) considered not only the mycoplasma etiology of the disease but also the similarity of the disease to yellow dwarf disease of rice.

Based on color picture of the diseased plants (Baldacci et al., 1970), the symptoms of giallume resemble tungro disease rather than yellow dwarf because the diseased plants have yellowing instead of general chlorosis and the number of tillers is slightly reduced rather than profusely increased. Therefore, giallume may not be identical with yellow dwarf. However, information on transmission and other characteristics are also needed for comparison.

GRASSY STUNT DISEASE

Grassy stunt disease of rice (Rivera, Ou, and Iida, 1966) and rice rosette (Bergonia et al., 1966) were simultaneously reported. From the information in these two publications, the diseases are not basically different in symptomatology, vector species, and virus-vector interaction. According to Rivera et al. (1966) the disease was first observed at the IRRI farm in 1963, and the transmission by *Nilaparvata lugens* was first demonstrated in 1964. This is verified by the information abstracted in the Review of Applied Mycology (Plant pathology, 1966) that was published earlier than either of the above-mentioned two papers. In other words, the name, grassy stunt was known to the public earlier. Consequently, grassy stunt is adopted for the name of the disease.

The disease may have occurred in the Philippines earlier than 1963 According to Bergonia et al. (1966), the disease was first observed in the 1959-60 planting season on a few plants at the Central Experiment Station, Bureau of Plant Industry, Manila. On the other hand, a drawing of a diseased rice plant in plate 4 in a paper by Agati, Sison, and Abalos (1941) looks like grassy stunt disease because of stunting, profused tillering, narrow leaf blades, and erect growth habit. If the drawing was accurate, it leads to the suspicion that grassy stunt disease may have existed in the Philippines for more than 30 years.



The disease has been observed in various parts of the Philippines. It seems more prevalent on farms that have an overlapping cropping system than in ordinary farmers' field. In addition to the Philippines, the disease has also been identified in Thailand (Wathanakul, Chaimangkol, and Kanjanasoon, 1968) and Ceylon (Abeygunawardena, Bandaranayaka, and Karandawela, 1970). It may have occurred in India (Raychaudhuri, Mishra, and Ghosh, 1967a) and Malaysia (Ou and Rivera, 1969).

The yield loss is determined by the plant age at the time of infection. When IR8 and Taichung Native 1 are inoculated at less than 30 days after sowing, practically no grain can be harvested. When inoculated at 45 days old, the yield reduction is 69 percent for IR8 and 77 percent for Taichung Native 1. When inoculated at 60 days old or older, the yield is not reduced significantly (Palomar and Ling, 1968).

Symptoms

When fully developed, symptoms on the diseased plants are severe stunting; excessive tillering, and an erect growth habit. The leaves are short, narrow, pale green or pale yellow, and often have numerous small, dark-brown dots or spots of various shapes which may form blotches. Young leaves of some varieties may be mottled or striped. The leaves may remain green when supplied with adequate nitrogenous fertilizer.

The rice variety, Shan-san-sa-san shows conspicuous striping when infected (Ling, Aguiero, and Lee, 1970). The stripes, one to several in number, are narrow, have diffuse margins, are yellowish-white, and are parallel to the midrib. They are either located at the basal portion of the leaf blade or they extend the whole length of the leaf blade.

The growth of the rice plant following infection is greatly arrested, the diseased plant becomes markedly stunted, while numerous diminutive tillers develop producing a rosette appearance. The infected plants usually live until maturity but they produce no panicles or a few, small panicles which bear dark brown and unfilled grains when infection occurs at early stages of plant growth.

The growth retardation is determined by plant age at the time of infection. The reduction in plant height of IR8 is 55% when inoculated at 15 days after germination, 43% at 30 days, 15% at 45 days, 10% at 60 days, and 1% at 75 days. For Taichung Native 1, the plant height reduction is 64% when inoculated at 15 days, 59% at 30 days, 14% at 45 days, and 2% at 60 days (Palomar and Ling,

Generally, the older the plants are when inoculated, the lower the percentage of diseased plants because rice plants infected at an old age may not develop symptoms before harvest. But the symptoms often occur on the regenerated growth when the plants are ratooned after harvest.

Transmission

Grassy stunt disease is only known to be transmitted by *Nilaparvata lugens* (Stål) (Rivera et al., 1966; Bergonia et al., 1966), commonly known as brown planthopper. None of 7,889 seedlings from 13,125 seeds harvested from diseased plants of several rice varieties developed symptoms. Hence, the disease apparently is not transmitted through seed (IRRI, 1968).

The causal agent of the disease persists in the insect vector. The proportion of active transmitters in field populations varies from 3 to 50 percent. Usually however, 20 to 40 percent are active transmitters. There are no consistent differences in percentage of active transmitters between male and female adults, between insects of dark brown and light brown color, or between macropterous and brachypterous forms. The incubation period in the insect is 5 to 28 days, average 10.6 days.

Most infective insects retain their infectivity until death; a few retain their infectivity for only a few days and become noninfective for the rest of their lives. The longest retention period obtained is 40 days. The insects acquire the causal agent at the nymphal stage and become infective at the adult stage after moltings without access to another disease source. Hence, transstadial passage of the causal agent in the insect occurs. The transmission pattern can be classified as intermittent rather than consecutive because more than 60 percent of the infective insects fail to transmit the disease consecutively at either hourly or daily intervals. The average number of disease-transmitting days is 0.81 during the period from the time the insect becomes infective until its death. That means that the infective insect transmits the disease on about 80 percent of days during that period. No infective nymphs have been obtained from eggs of infective females, hence, there is no evidence of transovarial passage of the causal agent.

The shortest inoculation feeding period is 9 minutes. Within 24 hours the longer the inoculation period is, the higher the percentage of positive transmission (Ling, Lee, and Aguiero, 1969). Rivera et al. (1966) reported however that the shortest acquisition feeding period is 30 minutes, the shortest inoculation feeding period minutes, and the incubation period in plant is 10 to 19 days.

Although brown planthoppers often confine themselves to the basal portion of rice plants, positive transmission is obtained by confining the viruliferous insects on the leaf blade of rice seedlings (Ling et al., 1969). The average life span of virus-free insects, 20.4 days, is significantly longer than that of viruliferous insects, 16.1 days. Furthermore, among the viruliferous insects, the average life span of infective insects, 15.4 days is significantly shorter than that of noninfective insects, 17.5 days (IRRI, 1968). Hence, the causal agent has a deleterious effect on the insect.

The causal agent

The causal agent of the disease is not clear at the present time. Virus particles of 70 nm in diameter have been observed in the ulrathin sections of infective insects (IRRI, 1966). But mycoplasma-like bodies have also been found in the diseased tissues (IRRI, 1968). The application of tetracyclines, which are sensitive antibiotics of mycoplasma, to diseased plants, to seedlings before and after inoculation, and to viruliferous insects did not cause striking differences from the controls (IRRI, 1968).

Host range

In addition to *Oryza sativa*, 15 species are also the hosts of the causal agent (Ling et al., 1970):

O. alta
O. australiensis
O. breviligulata
O. glaberrima
O. granulata
O. latifolia
O. minuta
O. nivara
O. officinalis
O. punctata
O. perennis
O. perennis subsp. *balunga*
O. rufipogon
O. sativa f. *spontanea*
O. spontanea (Australia)

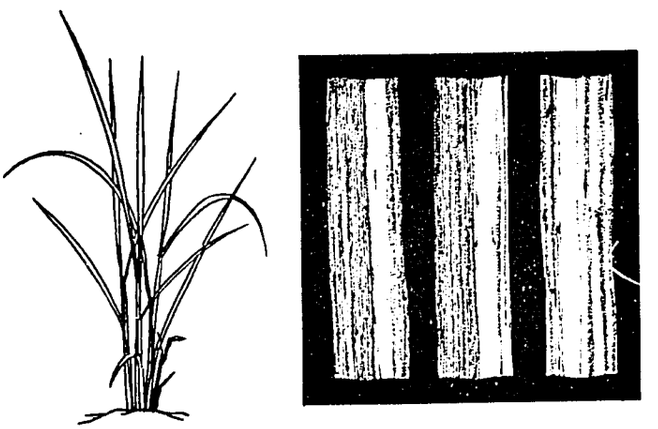
Varietal resistance

Khush (1970) reported that, based on field tests, the following varieties are tolerant to grassy stunt disease: BPI 76, Emata, H8, H105, HR35, Khao Dawk Mali 4-2-105, Khao Nam Kahang 92, Khao Pah 8-5-41, Khao Selti, Leuang Hawn, Niaw San Pah, Pah Leuad, Puang Nahk 16, Tawng, and TKM 6. Generally, 30 to 60 percent of the plants of these varieties did not show any disease symptoms in the test.

A method for testing varietal resistance to grassy stunt disease by artificial inoculation was developed in 1968 (Ling and Aguiero,

1968). By the end of 1970, more than 10,000 entries of rice varieties and lines were tested. Not a single tested rice variety consistently showed less than 30 percent infection. Fortunately, in 1969, a line of *O. nivara* was found to be highly resistant to the disease (Ling et al., 1970). The trivial name of this species was given by Sharma and Shastry (1965). Other lines of the same species tested were not resistant. The resistant gene from *O. nivara* has now been used by the breeders at IRRI for breeding resistant varieties. According to Khush (1970) resistance to grassy stunt in *O. nivara* is governed by a single dominant gene.

O. nivara is resistant to grassy stunt but susceptible to the vector, *N. lugens* (Ling et al., 1970). Since Mudgo is resistant to *N. lugens* (Pathak, Cheng, and Fortuno, 1969) but susceptible to grassy stunt (Ling et al., 1970), resistance to grassy stunt may not be associated with resistance to the vector.



Symptoms of hoja blanca disease.

HOJA BLANCA DISEASE

Hoja blanca (pronounced, o-ha blan-ka) is a Spanish name meaning white leaf. The name was given to the disease because of the white color of the foliage of infected plants. The disease is also known as chlorosis, cinta blanca (white band), raya (stripe), raya blanca (white stripe), and rayadilla (striped) in Latin American countries. The origin of the disease is obscure; however, it has been known to occur in Colombia since at least 1935 (Garcés-Orejuela, Jennings, and Skiles, 1958). It was not recognized as a serious disease of rice until 1956 (Hoja blanca, a threat to U.S. rice, 1957). The first successful studies to determine the viral nature of the disease were made by Malaguti, Diaz C., and Angeles (1957) in Venezuela. They reported that at least two vector species transmit the disease but they did not name them. Later it was learned that the species were the planthopper, *Sogatodes orizicola* and the leafhopper, *Hortensia similis*. However, Acuña Galé, Ramos-Lédon, and López Cardet (1958) were the first to report *S. orizicola* to be the vector of hoja blanca. *H. similis* has not been confirmed as a vector of the disease.

The disease is known to occur only in Western Hemisphere. Everett and Lamey (1969) reported that the disease has been found in Argentina, Brazil, British Honduras, Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Ecuador, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Peru, Puerto Rico, Surinam, the United States (Florida, Louisiana, and Mississippi), and Venezuela.

Hoja blanca causes slight to nearly complete yield losses, depending upon the extent of infection. Losses of 40 to 75 percent for individual fields have been common (Atkins and McGuire, 1958). For instance, in 1956, the growers in Cuba lost one-fourth of their crop; and in Venezuela, more than half (U.S. Department of Agriculture, 1960). Jennings (1963) developed a simple method to predict the yield loss prior to flowering based on the visual rating scale for leaf symptoms of 0 to 9, where 0 represents no disease; 9 is the severest attack possible. The percentage of yield loss is the value of the scale unit times 10.

Symptoms

The symptoms of hoja blanca disease as described by Atkins and Adair (1957): One or more white stripes on the leaf blade, or the entire leaf blade may be white, or the leaves may be mottled in a typical mosaic pattern. Diseased plants are reduced in height, and in severely affected fields the panicles of healthy plants are taller than those of the diseased plants. The panicles of diseased plants are somewhat reduced in size and often are not fully exserted from the sheath. The lemma and palea show a brownish discoloration and dry out rapidly, and frequently are distorted in shape. The floral parts are often absent, or if present they are sterile. As a result, panicles of diseased plants contain few or no seeds and remain in an upright position. Infected plants are not killed by the disease, and new tillers of a second or ratoon crop often show no symptoms. Both normal and diseased tillers frequently are observed on the same plant.

McGuire, McMillian, and Lamey (1960) pointed out that there are varietal differences in expression of symptoms, but in general, the disease can be easily recognized in all susceptible varieties. The severity of the symptoms, however, is inversely proportional to the age of the plant when inoculation takes place. The leaf on which the inoculation occurs may show a few chlorotic spots or be completely unaffected. An infected plant observed throughout its growing period develops the following symptoms: 1) The first leaf to emerge after inoculation, depending on the age of the plant, shows symptoms which vary from a few chlorotic spots at the base of the leaf to an extensive mottled or yellow-striped area which does not extend to the tip of the leaf. 2) The second leaf to emerge may exhibit general yellowing, mottling, or yellow stripes which run the whole length of the blade. 3) The third leaf to emerge usually is completely chlorotic and often dies before the other leaves. Necrosis begins at the tip and upper edges and progresses downward and inward.

Young plants when infected show the complete progression of the disease, whereas older plants do not show the more advanced symptoms. If infection occurs before or during the booting stage, the panicle may not completely emerge and all or some of the spikelets may fail to set seed. If the infection occurs after the emergence of the panicle, only a small reduction in seed production may occur.

Plants infected at about the five-leaf stage are reduced in height by about 56 percent. Extreme reduction may occur since the younger leaves die sooner than those preceding them and the emerging leaves are stunted. In the field rice plants seem to recover from the disease periodically. However, the recovery is in the appearance of the field not in the individual plant, which remains diseased until death or maturity.

Gálvez E. (1969a) mentioned that in general, the culms are mottled and the infected plants may die if the plants are infected at a very early stage of growth.

Susceptibility of seedling to the disease varies according to the seedling age. The results obtained by Lamey, Everett, and Brister (1968) indicated that the percentage of infected seedlings of seven tested varieties decreased when the age of seedlings at the time of inoculation increased from the one-leaf to the three-leaf stage.

Hoja blanca virus infection does not affect the lesion development of the rice blast fungus, *Pyricularia oryzae* but enhances the lesion development of brown spot caused by *Cochliobolus miyabeanus* (Lamey and Everett, 1967).

Transmission

Hoja blanca is known to be transmitted by two species of *Sogatodes: S. orizicola* (Muir) and *S. cubanus* (Crawford). The latter was first reported to transmit the disease from rice to *Echinochloa colonum* and from *E. colonum* to *E. colonum* but not from rice to rice or from *E. colonum* to rice (Gálvez, Thurston, and Jennings, 1960). Later, however, Gálvez (1968b) was able to effect transmission from rice to rice and from *E. colonum* to rice by *S. cubanus*. *S. orizicola* is the major vector of rice hoja blanca because it prefers rice. On the other hand, *S. cubanus* may play an important role in the disease cycle under natural conditions although it cannot live on rice for any length of time.

Although several other species of insects *Aphis maidis, Draeculacephala portola, Graminela nigrifrons, Hortensia similis, Peregrinus maidis* and *Rhopalosiphum maidis*, and the mite, *Tetranychus* sp., have been tried for the transmission of the disease, none gave confirmed positive transmission (McGuire et al., 1960). No

evidence has been found for transmission of the disease by mechanical means, through soil, or through seeds (Gálvez E., Jennings, and Thurston, 1960). More than 8,500 seeds from infected plants have been tested by different investigators and none has obtained a diseased seedling (Gálvez E., 1969b).

The biological relationship between the virus and the vector is not completely clear. However, the virus not only persists in *S. orizicola* but also is transmitted through the eggs from infective females of *S. orizicola* to their progenies. For *S. orizicola,* most investigators agree that 5 to 15 percent of the field population are active transmitters. The percentage of active transmitters can be increased by selective breeding. The shortest acquisition feeding period is 15 minutes (Gálvez E., 1969b). The incubation period in the insect is reported to range from as little as 5 days in some publications up to 37 days in other publications. Gálvez (1968b) emphasized, however, that the incubation period is 30 to 36 days so that the male insect can only transmit virus it has acquired congenitally from the female parent because the average life span of male insect is shorter than 30 days. Hendrick et al. (1965) reported that an infective insect does not necessarily transmit the disease every day. But, Gálvez E. (1969b) found that the insect transmits the disease every day without any irregularity. The longest retention period reported is 14 days (Hendrick et al., 1965) which is the longest time duration tested.

The existence of transstadial passage and transovarial passage has been proved by various investigators. Transovarial passage was first demonstrated by Acuña and Ramos in 1959 (Gálvez E., 1969b). Depending on which publication is used as an authority, 60 to 94 or nearly 100 percent of the insects are congenitally infective. Gálvez E., (1969b) reported that the virus apparently can pass through 10 successive generations without diminishing in concentration. On the other hand, in 1966, W.B. Showers (Everett and Lamey, 1969) observed that the congenitally infective insects failed to transmit the disease to a series of six rice seedlings. Showers also found that nymphs from infective female may transmit the disease within 24 hours of hatching.

The shortest inoculation feeding period is 30 minutes (Gálvez, 1968b). The incubation period in plant varies from 3 or 4 to 45 days (Gálvez E., 1969a) according to the seedling age at the time of inoculation, susceptibility of the rice variety, and different publications.

Virus-free *S. orizicola* laid twice as many eggs as viruliferous individuals, according to W.B. Showers (Maramorosch, 1969).

Similarly, Showers and Everett (1967) reported that the life span of adult insects of progeny of an infective female is significantly shorter than that of progeny of an infective male. Recently, Jennings and Pineda T. (1971) reported that viruliferous insects lay one-third as many eggs and hatch fewer nymphs than do virus-free insects. The percentage of nymphs reaching the adult stage and the life span of the insect are also reduced. These findings indicate that the virus has deleterious effects on *S. orizicola.*

Little is known about the virus-vector interaction for *S. cubanus.* Gálvez (1968b) pointed out that using at least 10 insects per seedling, the insect can transmit the disease from rice to rice (10%), from *E. colonum* to rice (25%), and from *E. colonum* to *E. colonum* (80%). On the other hand, G. Granados (Everett and Lamey, 1969) found that 23 percent of a field population of *S. cubanus* collected at Cotaxtla, Mexico, transmitted the virus to healthy seedlings of *E. colonum.* Eighty percent of the insects, however, transmitted the virus after feeding on diseased plants.

The virus

The description of the virus particles of hoja blanca is unsettled since the only two publications on the subject disagree. Herold, Trujillo, and Munz (1968) reported that the virus particles are spherical and approximately 42 nm in diameter in dip preparations and in purified suspensions from the diseased leaves. In contrast, Shikata and Gálvez E. (1969) reported that numerous bundles of long, flexuous, threadlike particles are found in cells of both the diseased leaves and the infective insects. The threadlike particles are approximately 8 to 10 nm in diameter and variable in length. They appear in the epidermis, palisade cells, spongy parenchyma, phloem, and vessels of the diseased leaves. In the plant cells, the threadlike particles appear in the nuclei and cytoplasm, but not in the chloroplasts or mitochondria. In the insects, the threadlike particles are found in the lumen and epithelial cells of the intestine, probably in the filter chamber. These threadlike particles may be the virus of hoja blanca.

The purification procedure used by Herold et al. (1968) is as follows: One part of diseased leaves (2 to 6 g), six parts of 0.5 M citrate buffer pH 6.5 (containing 0.1% of thioglycolic acid) and three parts of chloroform (w/v) are homogenized and squeezed through nylon cloth. The resulting liquid is then centrifuged at 5,000 *g* for 15 minutes. The supernatant liquid is dialyzed in 0.005 M borate buffer, pH 9.0, for 15 hours and concentrated by ultracentrifugation

at 105,000 *g* for 90 minutes. The pellet is resuspended in 0.3 to 1.0 ml of borate buffer containing 250 units of penicillin and 250 μg/ml of streptomycin. This is the virus preparation.

The infectivity of the preparation is tested by injecting 0.2 microliters of the preparation into *S. orizicola* nymphs at the last instar. The injected insects are maintained on healthy rice plants during an incubation period of 11 days. Then the insects are caged singly on rice seedlings at one-leaf stage for inoculation. By this method, Herold et al. (1968) obtained three out of 36 injected insects that transmitted the disease while none of the 42 insects injected with the corresponding suspension of healthy leaves did.

Host range

Since Malaguti (1956) reported hoja blanca on *Echinochloa* sp. in the field, the following grasses, as compiled by McGuire et al. (1960) have been reported as showing symptoms similar to those of hoja blanca in rice:

Brachiaria plantaginea
Echinochloa colonum
E. crusgalli
E. walteri
Echinochloa sp.
Panicum fasciculatum
P. capillari
Panicum sp.
Paspalum sp.
Rottboellia exaltata
Sacciolepis striata

red rice, and black rice. Later, Gibler, Jennings, and Krull (1961) reported that of hoja blanca occurred naturally on several varieties of wheat and oat and hybrids involving crosses with *Triticum aestivum* and *T. durum*.

Since Acuña and Ramos (Gálvez E., 1969a) infected *E. colonum* with viruliferous *S. orizicola*, several species of plants have been infected artificially by various investigators. Gálvez, Thurston, and Jennings (1961) infected *Avena sativa*, *Digitaria horizontalis*, *Hordeum vulgare*, *Leptochloa filiformis*, and *Triticum aestivum*. Later, Lamey, McMillian, and Hendrick (1964) added *Secale cereale* and *Triticum compactum*, and Gálvez (1968b) added *Cyperus* sp. to the list.

Varietal resistance

In 1957, 2,200 rice varieties and selections were tested for resistance to hoja blanca under natural conditions in Cuba and Venezuela. Atkins and Adair (1957) found 540 that were resistant. All the U.S. long-grain varieties and the commonly grown short-grain varieties were susceptible. However, several minor U.S. short-grain and

medium-grain varieties such as Asahi, Colusa, Lacrosse, and Missouri R-500 were resistant. Later, in Colombia, Lamey, González, Rosero, Estrada, Krull, Adair, and Jennings (1964) found that Asahi, Colusa, Gulfrose, Lacrosse, Lacrosse x C253, Lacrosse x Zenith-Nira, and Pandhori No. 4 were resistant.

A greenhouse method for testing varietal resistance to hoja blanca was developed by Lamey, Lindberg, and Brister (1964). They found Arkrose, Berlin (PI 202864) and Gulfrose showing less than 5 percent infection. Gulfrose and Nova seem to be the most resistant (Lamey et al., 1968) because they showed less than 30 percent infection even when inoculated at the one-leaf stage.

Based on the reaction of single-cross F_1 plants, backcross F_2 plants, and F_3 and F_4 line selections, Beachell and Jennings (1961) concluded that the resistance is dominant and controlled by one major gene pair. However, modifying genes may be present that influence the degree of resistance of a variety.

Resistance to hoja blanca is not necessarily associated with resistance to *S. orizicola*. Jennings and Pineda T. (1970) reported that Bluebonnet 50 is susceptible to the insect and to the disease, IR8 is resistant to the insect and susceptible to the disease, ICA-10 is susceptible to the insect and resistant to the disease, and Mudgo is resistant to both the insect and the disease.

NECROSIS MOSAIC DISEASE

Rice necrosis mosaic refers to necrotic lesions that form on culms and mosaic mottling that forms on the leaf blades of the diseased rice plant. It does not mean that the mosaic mottling is necrotic. The name in English first appeared in the literature in 1968 (Inouye, 1968). But, it has been known in Kana (Japanese), as "eso (necrosis) mosaic," since 1967 (Fujii et al., 1967). Before 1967, the disease was tentatively called "yaika-sho" (dwarfing symptom) (Fujii et al., 1966). The first record of the occurrence of the disease was in Okayama Prefecture, Japan in 1959 (Fujii, 1967). The disease has been found in the following Prefectures of Japan: Hyogo, Kagawa, Oita, Okayama, and Shizuoka (Fujii and Okamoto, 1969; Fujikawa, Tomiku, and Sato, 1969). In 1964, 269 hectares in Okayama Prefecture were diseased (Fujii, 1967). In severely affected areas 47 percent of the grain yield was lost (Fujii, 1967).

Symptoms

The major symptoms of necrosis mosaic are spreading growth habit, mosaic mottling on the leaf blades especially of the lower leaves, and necrotic lesions on basal portions of the culms and leaf sheaths. Under natural conditions, the infection occurs mostly in upland seedbeds.

The first symptoms usually appear as mosaic mottling on the lower leaves at about the maximum tillering stage after transplanting in the paddy field. The mottling consists of streaks, light green to yellow, oval to oblong, about 1 mm in width, 1 mm to more than 10 cm in length. Later, the streaks may coalesce to form irregular

Symptoms of necrosis mosaic disease

patches. When many patches appear on a leaf, the leaf turns yellow. The patches or mottling gradually spread to the upper leaves. The mottling may occur on a part of culm.

The reduction in plant height is not very conspicuous. The reduction varies from 2 to 12 percent according to the severity of the disease. The number of tillers is reduced. The tillers tend to lie flat at their bases, resulting in a spreading growth habit. At later stages, a few, elongated, irregular, brownish necrotic lesions appear on the surface of leaf sheaths as well as on basal portion of culms, particularly of the main and primary tillers. The infected plants often survive until harvest. But they produce fewer panicles with fewer grains. The grains have a lighter weight (Fujii, 1967; Fujii and Okamoto, 1969).

X-bodies which are round to oval, 4.3 to 11.9 by 7.1 to 26.9 microns, are present in the cells of the inner epidermis of leaf sheath of the diseased plants. The bodies serve as a way to diagnose the disease. They can easily be seen when the epidermal layer is peeled off from the leaf sheath, stained with iodine solution, and examined under a microscope. (Fujii, 1967; Fujii and Okamoto, 1969).

Based on field observation, the infected plants are more susceptible to blast disease (Fujii, 1967).

Transmission

Evidence that insects or seeds transmit the disease has not been found. But the disease can be transmitted by mechanical means and through soil.

The transmission of the disease by mechanical means was first reported by Fujikawa et al. (1969). They prepared the inoculum by grinding diseased leaves in sterile water at 10 ml per 1 g leaf. The sap was then inoculated into the middle portion of leaves of healthy rice plants by rubbing them with carborundum. The first symptom appears as gray streaks of 1 by 2 to 3 mm on the inoculated portion 5 to 7 days after inoculation. About 2 weeks later, streaks develop on the upper portion of the inoculated leaves. Sometimes, it takes 30 to 50 days for the inoculated plants to show the symptoms. When plants are inoculated by this method, 40 to 70 percent become infected.

The transmission of the disease through soil was first demonstrated by Fujii et al. (1968) in 1966. And it was confirmed by Fujikawa et al. (1969). The seedlings grown in pots with soil collected near diseased plants in the field become infected under unflooded

paddy field after 47 days in pots or remain in pots. In contrast in pots containing the same soil sterilized by heat at 57 to 60 C for 30 minutes or longer, seedlings remain healthy. The symptoms often take 2 months or longer to appear and 50 to 100 percent of the plants become infected (Fujikawa et al., 1969).

The virus

The virus particles of necrosis mosaic are rod-shaped, with two length-distribution peaks, 275 and 550 nm. They are 13 to 14 nm in diameter when dip preparations from roots of diseased plants are examined under an electron microscope. The particles are usually present in the dip preparations from leaf blades, leaf sheaths, and roots of natural infected plants and seedlings grown in soil collected around diseased plants in the field (Inouye, 1968).

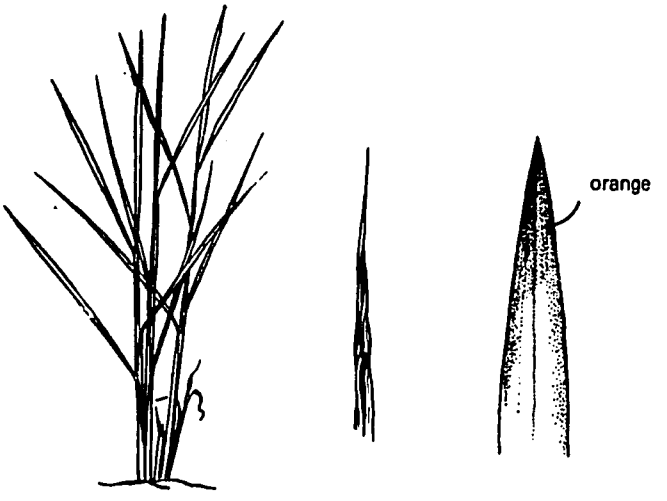
When the infectivity of the virus is determined by mechanical inoculation, the dilution end point is 5×10^{-3} to 10^{-4}. The thermal inactivation point is 60 to 65 C for 10 minutes. The virus remains infectious at 20 C for 7 days but not for 14 days (Fujikawa, Tomiku, and Sato, 1970).

Varietal resistance

Under natural conditions, the rice varieties Shinonome-mochi and Omachi were resistant to the disease. Akebono, Asahi, Nakate-shin-senbon, Nishikaze, and Yamada-nishiki were moderately resistant (Fujii and Okamoto, 1969).

Control

Since the virus is soil-borne and infection often occurs in upland seedbeds, diseased seedbeds should not be re-used. Raising seedlings in wetbed nurseries and sterilizing the soil of the seedbed with heat or chemicals can also help prevent the disease.



Symptoms of orange leaf disease.

ORANGE LEAF DISEASE

The first record of the occurrence of the disease was made by Ou (1963) in Thailand in 1960. It was observed in the Philippines in 1962 and a year later it was identified as a new virus disease of rice by Rivera, Ou, and Pathak (1963). The disease has also been identified by symptomatology and transmission in Thailand (Wathanakul, Chaimangkol, and Kanjanasoon, 1968) and in Ceylon (Abeygunawardena, Bandaranayaka, and Karandawela, 1970), and it has been observed in Malaysia (Ou and Rivera, 1969) and in India (Pathak et al., 1967).

The diseased plants often are scattered through the field and diseased fields are sporadically distributed. The disease does not cause serious losses in yield although the infected plants often die prematurely.

Symptoms

In the field, the diseased plants have golden yellow to deep bright-orange leaves when the plants are about 1 month old or older. Later, these discolored leaves gradually roll inward and dry out starting from the tip. The infected plants die before flowering. If a

rice hill is originally from a few seedlings, not all tillers will necessarily be infected; some may remain normal.

The first symptoms on plants inoculated artificially appear on the outer margin or on only one side of the leaf blade near the tip as one or well-defined orange stripes which run along the veins. Later, the leaves rapidly turn bright orange to nearly yellowish and roll inward beginning at the leaf tip and then progressing downward. Infection slows the plant's growth, but it does not stunt growth. Nevertheless, the plant produces fewer tillers, and the roots develop poorly.

Infected plants die quickly especially when they are inoculated at an early stage of growth. When plants are infected at a later stage of growth, panicles may develop but may not emerge properly from the sheath; moreover, the grains are often unfilled (Rivera et al., 1963).

Transmission

Orange leaf is transmitted by *Recilia dorsalis* (Motschulsky) (Rivera et al., 1963; Wathanakul et al., 1968; Abeygunawardena et al., 1970), commonly known as the zigzag leafhopper. Tests have failed to show the transmission of the disease through soil or seeds (200 seeds from infected plants tested), or by mechanical means with the sap of diseased plants (Rivera et al., 1963; Wathanakul et al., 1968), or by insects such as *Macrosteles fascifrons, Nephotettix apicalis, Nisia atrovenosa, Peregrinus maidis, Sogata paludum, Tettigella spectra* (Rivera et al., 1963) and *Nephotettix* sp. (Wathanakul et al., 1968).

The virus seems to be persistent in the vector. The proportion of active transmitters varies from 7 to 14 percent. The shortest acquistion feeding period is 5 hours. The incubation period in the insect is 2 to 6 days (Rivera et al., 1963). But Wathanakul et al. (1968) reported that virus-free insects acquire the virus in a 1-day feeding period, and the following day transmit the virus in a 1-day inoculation feeding. Once the insects become infective, they seem to retain their infectivity until death (Rivera et al., 1963). In serial transmission studies, Abeygunawardena et al. (1970) reported that a single viruliferous vector is capable of infecting over four plants in succession without having to feed on a fresh virus source. The shortest inoculation feeding period is 6 hours. The incubation period in plant is 13 to 15 days (Rivera et al., 1963). Wathanakul et al. (1968) reported however that symptoms appear in most plants 14 to 21 days after caging with viruliferous insects.

The virus

The electron micrographs of ultrathin sections of viruliferous *R. dorsalis* made at the Boyce Thompson Institute for Plant Research revealed that the virus particles are spherical and 15 nm in diameter. The result has not been confirmed or published, however.

Host range

Wathanakul (1964) inoculated 29 species of plants with viruliferous insects, none became infected; nor could virus-free insects recover the virus from the inoculated plants and transmit it to rice plants.

Varietal resistance

Abeygunawardena et al. (1970) tested 32 rice varieties for their resistance to orange leaf by inoculating the seedlings at the two-leaf to three-leaf stage with viruliferous insects for 24 hours. They found that Kalu Dahanala had only 20 percent infection while others had 40 to 100 percent infection. The infected seedlings of Kalu Dahanala show mild symptoms of leaf yellowing but no leaf rolling or death of seedling. Hence, the variety is not only resistant to orange leaf disease but also tolerant.

Symptoms of stripe disease.

STRIPE DISEASE

Stripe disease is a translation of Japanese "shima-hagare-byo" which literally means striped leaf blight. The disease has been known in Gunma, Tochigi, and Nagano, Japan since the 1890's (Shinkai, 1962). The earliest recorded major outbreak of the disease in Nagano Prefecture was in 1903 (Kuribayashi, 1931). Kuribayashi (1931) was the first to demonstrate the transmission of the disease by the insect vector, *Laodelphax striatellus*. The disease is widely distributed in Japan except in Hokkaido and the northern parts of Tohoku (Iida, 1969). Since 1953 when earlier rice planting was generally adopted in Japan (Iida, 1969), the disease has become a serious problem because the vector not only propagates more easily in the early-set nursery and the early transplanted field but it also can transmit the virus more easily (Ishikura, 1967).

The disease is also widespread in South Korea (Lee, 1969). It may occur in Taiwan, too.

The reduction in yield due to the disease is determined by plant age at the time of infection. Rice plants produce no grain when they are inoculated earlier than the 11-leaf stage. The yield reduction gradually decreases, the older the plants are at infection. When rice plants are inoculated later than the 13-leaf stage, the reduction becomes negligible (Shinkai, 1962).

Symptoms

The symptoms of stripe disease, as described by Iida (1969) are characterized by the failure of emerging leaves to unfold properly. Some leaves emerge without unfolding, then elongate and become twisted and droop. They lack vigor and show general chlorosis with, often, a wide chlorotic stripe pattern with diffuse margins. A gray necrotic streak frequently appears in the chlorotic area, which enlarges and kills the leaf. Other leaves, especially those which emerge later during growth and unfold properly, show an irregular chlorotic mottling that often appears in a stripe pattern along the length of the blades. Mottling may also appear on the leaf sheath.

When infection occurs at early stage of plant growth, the entire plant may die prematurely, or be considerably stunted. In plants infected later, stunting may be slight. The tiller number of infected plants is usually highly reduced.

Diseased plants produce a few poor panicles, if any, which carry characteristically malformed spikelets and which have difficulty emerging from leaf sheaths. Probably due to late infection, some plants which have shown no leaf symptoms until maturity produce panicles carrying many malformed and unfilled grains.

On more resistant varieties, chlorotic mosaic mottling on leaves is the only symptom. On the other hand, rice plants inoculated at the 13-leaf stage or later, often show no symptoms at all before harvest (Yasuo, Ishii, and Yamaguchi, 1965).

Kawai (1939) found X-bodies, 1.5 to 3.5 by 1.5 to 4.5 microns, in the mesophyll cells and sometimes in the motor cells, near the nuclei of affected plants. When the inside epidermis of a leaf sheath is examined, large, eight-figured, ring-shaped, round-shaped, and rod-shaped inclusions are present (Hirai et al., 1964; Kashiwagi and Sasaki, 1966). The inclusions generally contain many granules, but some have no granules and resemble crystalline inclusions. Corner inclusions are also present. Sometimes, chloroplasts in the leaf blade degenerate, which may result in the formation of yellow lesions. In such cells, small needle crystals are embedded in the degenerated chloroplasts.

Transmission

Stripe disease is now known to be transmitted by *L. striatellus* (Fallén) (Kuribayashi, 1931), *Unkanodes sapporonus* (Matsumura) (Shinkai, 1966), and *Ribautodelphax albifascia* (Matsumura) (Shinkai, 1967; Hirao, 1968b). No evidence has been obtained to demonstrate that the disease can be transmitted through seeds or

by mechanical means (Kuribayashi, 1931). Okuyama and Asuyama (1959) reported, however, that they could inoculate seedlings with virus by injecting the sap of diseased leaves into the midrib of the rice leaves. Others have not reproduced this result.

The virus multiplies in the insect vectors as shown by evidence of transovarial passage (Yamada and Yamamoto, 1955) and by serial transfer of the virus from insect to insect by injection (Okuyama, Yora, and Asuyama, 1968). The proportion of active transmitters of *L. striatellus* is 14 to 54 percent (Kuribayashi, 1931) and of *R. albifascia*, 28 to 35 percent (Shinkai, 1967). The shortest acquisition feeding period is 3 minutes for *L. striatellus* (Yamada and Yamamoto, 1955) and less than 30 minutes for *R. albifascia* (Hirao, 1968b). The incubation period of the virus in *L. striatellus* is 5 to 21 days, mostly 5 to 10 days (Shinkai, 1962), and in *R. albifascia*, 5 to 26 days, with an average of 12 days (Hirao, 1968b). Transstadial passage of the virus exists and the virus persists in the vector. The longest retention period of *L. striatellus* is 47 days (Shinkai, 1962). Transovarial passage occurs in all the three species of vectors. For *L. striatellus*, 42 to 100 percent of the insects are congenitally infective; for the other two species, the percentage is high (Shinkai, 1966, 1967; Hirao, 1968b). The virus from a single infective female of *L. striatellus* can be passed through eggs to high proportion of the progeny in 40 succeeding generations over a period of 6 years (Shinkai, 1962). There is no progressive decline of the virus in the insects. Congenitally infective insects transmit the virus immediately after hatching (Shinkai, 1962). The incubation period in plants ranges from 10 to 25 days (Shinkai, 1962). The period becomes longer when the older plants are inoculated (Yasuo et al., 1965).

Temperature affects the transmissive ability of *L. striatellus*. When the insects have been reared at 25 C, the ability of the insects to acquire the virus at 10 C or below is lower than at 15 C or above. Insects reared at 10 C or below are unable to transmit the disease at 25 C than those reared at 15 C or above (Yasuo et al., 1965). Hirai et al. (1968) pointed out that about 50 percent of viruliferous insects that had fed on rice plants treated with Blasticidin S lost the capability for transmitting the disease as did a fairly large percentage of the progeny of parents that had fed on the treated plants. Possibly the compound acts on the virus in the insects and reduces the frequency of transovarial passage of the virus.

Rice stripe virus has been reported to be deleterious to *L. striatellus* (Nasu, 1963). But Kisimoto (Maramorosch, 1969) pointed out that no significant differences have been found in *L. striatellus* egg mortality or in the life span of insects hatched from eggs with or without the virus.

The virus

Saito, Inaba, and Takanashi (1964) reported that the particles of the stripe virus are spherical and 29.2 nm in diameter. Kitani and Kiso (1968) found that the size is 25 to 35 nm in diameter, mostly 30 nm.

The purification procedure for the virus as used by Kitani and Kiso (1968) is as follows: 100 g diseased leaves are homogenized with 200 ml of 0.01 M phosphate buffer, pH 7.2, squeezed through cheesecloth, and centrifuged at 5,000 rpm for 20 minutes. The chloroform is added to the supernatant liquid to make a 20 percent solution and centrifuged at 5,000 rpm for 20 minutes. The top layer, after polyethylene glycol (PEG) (Carbowax 6000) and NaCl are added to make 8 percent PEG and 0.43 percent NaCl, is shaken and centrifuged at 24,000 rpm for 60 minutes at 0 C. The pellet is resuspended in phosphate buffer and centrifuged at 5,000 rpm for 20 minutes. The supernatant liquid is then subjected to density-gradient centrifugation at 30,000 rpm for 180 minutes. The band at the 30 to 40 percent sucrose zone is removed and dialized against 0.01 M phosphate buffer. Then the PEG and NaCl step is repeated. The pellet is resuspended in phosphate buffer and applied to the DEAE-cellulose column, and eluted from the column with a linear gradient system (0.01 M phosphate buffer, from 0 to 1.0 M NaCl). The resulting suspension is dialized against phosphate buffer at 0 to 5 C. The PEG and NaCl step is repeated once more. The pellet is resuspended in 0.05 M tris buffer, pH 7.0. The purified virus suspension is obtained after the suspension has been centrifuged at 4,000 rpm for 10 minutes.

The infectivity of the virus preparation is determined by injecting the preparation into the insects. The infectivity of the insects is determined by seedling inoculation test. Kitani and Kiso (Suzuki and Kimura, 1969) pointed out that the dilution end point for the sap of diseased rice leaves is 10^{-3} and for sap of viruliferous insects 10^{-4}. The thermal inactivation point is 55 C for 3 minutes. The purified virus remains infectious for 1 month at −20 C. When viruliferous insects and diseased leaves are stored at −20 C, the virus remains infective up to 8 months.

The hemagglutination technique for detecting viruliferous insects and expressed sap of diseased leaves was developed by Yasuo and Yanagita (1963). The principle of the technique is that sheep erythrocytes treated with a dilute solution of tannic acid adsorb protein. Such protein-coated red blood cells are agglutinated by a specific antiserum to counteract the protein used for adsorption. When the blood cells are coated with a certain virus, the cells are agglutinated by the virus-specific antiserum (hemagglutination test

with antigen-sensitized cells). Conversely, when the cells are coated with the antiserum, the coated cells are agglutinated with virus (hemagglutination test with antibody-sensitized cells) (Suzuki and Kimura, 1969). Kitani, Kiso, and Yamamoto (1968) applied the method of direct staining the virus with fluorescein-labeled antibodies to locate the virus in *L. striatellus*. They found that the virus antigens are more concentrated in salivary glands, intestine, fat bodies, and ovarioles than in other tissues or organs such as the brain and mycetomes of the Malpighian tubes.

There are two strains of the stripe virus, the "curling type" and the "opening type" (Ishii and Ono, 1966). With the former strain, the newly emerged leaves remain folded. The latter, on the other hand, causes only mosaic symptoms. Both strain are usually found together in the same plant or insect in the field.

Host range

In addition to rice, 36 species of plants, as compiled by Iida (1969), are the hosts of the stripe virus:

Agrostis alba
Alopecurus aequalis
A. japonicus
Avena sativa
A. fa .
Beckmannia syzigachne
Briza minor
Bromus catharticus
Cynodon dactylon
Cynosurus cristatus
Dactylis glomerata
Digitaria adscendens
D. violascens
Echinochloa crusgalli
E. crusgalli var. *frumentacea*
Eragrostis multicaulis
Glyceria acutiflora
Hordeum sativum var. *hexastichon*
H. sativum var. *vulgare*
Leersia oryzoides var. *japonica*
Lolium multicaulis
L. perenne
Panicum miliaceum
Pennisetum alopecuroides
Phleum pratense
Poa annua
Saccharum koenigii
Secale cereale
Setaria italica
S. viridis
Sorghum halepensis
S. sudanense
Trisetum bifidum
Triticum aestivum
Zea mays
Zoysia japonica

The symptoms on most of these plants are rather mild, consisting of chlorotic streaks or mosaic mottling. In Japan, infected *D. adscendens*, *D. violascens*, *E. multicaulis*, *S. italica*, and *S. viridis* are frequently found in the field.

In Korea, several gramineous plants including barley, foxtail, Italian ryegrass, milkvetch, and wheat have been found infected by the virus (Lee, 1969).

Varietal resistance

From natural infection tests in Japan, Yamaguchi, Yasuo, and Ishii (1965) found several varieties that were highly resistant to stripe disease: Akula, Carolina, Central Patna 231, Co 13, Dahrial, Danahara, Kaladumai, Kaluheenalii, Karalath, Kentannangka, Kuan-yin-sien, Loktjan, Mao-tzu-tou, Pi-rih-tao, Tadukan, Tchelai, Tetep, Wu-chien, Wu-ku, and all varieties of Japanese upland rice. Later, Sonku and Sakurai (1967) tested 121 varieties and found 49 of them were highly resistant. In Korea, Jung et al. (1965) found that Nam Sun No. 94 and Nam Sun No. 111 were highly resistant.

A seedling test method for varietal resistance to stripe disease by artificial inoculation was developed by Sakurai, Ezuka, and Okamoto (1963).

By using this method, Sakurai and Ezuka (1964) found many resistant varieties, such as

Altai	Lua Rong
Ask Kata	Modan
Chitrai	Nep-Vai (gl.)
Danahara	P.T.B. 10
Hatadavi	Peta
Hsin-pa-sien	Russia No. 35
Intan	Russia No. 41
Karalath (H-32)	Russia No. 60
Karalath (H-33)	Tadukan
Kota	Tjahaja
Latisail	Tsao-sien-tao

In Korea, 410 varieties were tested by artificial inoculation. The following varieties were found to be resistant (Lee, 1969):

Arkrose	Shin No. 2
Gulfrose	St. No. 1
Li Chan Chil Il Chal	St. No. 2
Nong Lim No. 1	Sun Bonnet
Nong Lim No. 24	Yang Ju Bat Chal
O Baik Jo Ge	Zenith (Lee, 1969)

Kim et al. (1969) also reported the following varieties were resistant: Chugoku 31, IR9-6, Norinmochi 1, Norinmochi 26, Tadukan, Tetep, and Usen.

Based on the seedling reaction of hybrids between resistant Japanese upland rice varieties, Hatanishiki, Kanto Mochi No. 70, Kuroka, and Yukara, the resistance to stripe in Japanese upland rice varieties is controlled by two pairs of complementary dominant genes, St_1 and St_2 (Washio et al., 1967). Based on five crosses

between stripe resistant varieties, Zenith, Surjumkhi, Charnack, Russia No. 35, and Ketan-Nangka, and a susceptible Japanese paddy variety, Kibiyoshi, resistance in Zenith is controlled by two pairs of complementary dominant genes, suggesting some relation with Japanese upland rice. Resistance in other four varieties is incompletely dominant and controlled by one major gene, St_3. It seems that the action of the resistant gene, St_3, differs among varieties (Washio et al., 1968).

Resistance to the stripe virus may not always be associated with resistance to the vector, *L. striatellus*. Okamoto and Inoue (1967) reported a field test in which varieties Nep-Vai and Rikuto-Norin No. 11 had large numbers of insects, but few diseased plants. In contrast, there were few insects on variety Konanso but many diseased plants occurred. Their results showed the resistance to the virus and the resistance to the vector are independent. Based on the reactions, rice varieties can be grouped as 1) resistant to both the virus and the vector, such as Tadukan; 2) resistant to the virus but susceptible to the vector, such as Rikuto-Norin No. 11; 3) susceptible to the virus but resistant to the vector, such as Hu-nan-tsao; and 4) susceptible to both the virus and the vector, such as Asahi.

TRANSITORY YELLOWING DISEASE

Transitory yellowing is a name given by Chiu et al. (1965) to the disease because following an acute stage of leaf yellowing, diseased plants seem to recover gradually producing no yellow leaves at later stages of growth. The disease is known in Chinese as "huang-yet-ping" which means a disease of yellow leaves. Previously, Miu (1964) called it "brown-wilt". The disease may have existed in Taiwan for a long period but, it did not attract attention until 1960 when the disease broke out in southern Taiwan. Because of similarities in symptomatology, the disease was first confused with "suffocating" disease, which has been known for decades to be associated with a soil problem in northeast Taiwan. In 1965, however, Chiu et al. gave a report on the disease and its transmission by *Nephotettix apicalis*. At present, the disease is only known to occur in Taiwan.

The yield of diseased plants is 68 to 75 percent of the healthy plants. The loss is mainly due to fewer and smaller panicles and a higher percentage of unfilled grains (Hsieh, Wu, and Su, 1968).

Symptoms

Transitory yellowing and tungro are similar in some aspects. The characteristic symptoms of transitory yellowing consist of yellowing of leaves, reduced number of tillers if the plants are infected in an early growth stage, and stunting of the plants. The discoloration of the leaves starts usually from the distal portion of the lower leaves. Therefore, the color is more intense in the lower leaves than in the upper ones. Brown rusty flecks or patches may appear on the discolored leaves. Yellowing of the leaves varies among rice varieties, however. Yellowing may be slight or indistinct in the lower leaves which soon roll and wither. Only one or two uppermost leaves may live. The infected plants have a poor root system compared with the healthy plants. The plants infected early produce no panicles or poor ones.

Diseased plants often recover somewhat under greenhouse conditions. Following an acute stage of leaf yellowing for about a month or so, the infected plants may gradually recover and produce normal leaves at the later growth stages. Consequently, the appearance of the diseased plants may become normal after the leaves which had previously shown yellowing fall off (Chiu et al., 1965).

Based on the iodine test and chemical analysis, starch accumulates in the leaf blades of diseased plants. This does not happen in

leaf blades of plants with "suffocating" disease. Therefore, dipping the basal portion of the leaf blades in 0.6 percent iodine solution immediately after sampling has been suggested as a way to distinguish transitory yellowing from the "suffocating" disease in the field (Hsieh, 1966).

Large round inclusion bodies are found in parenchyma cells surrounding the xylem vessels and sieve tubes in the leaves and roots of diseased plants. These bodies are cylindrical and vary in length and thickness in longitudinal sections. Some of them occupy most of a cell. They consist of homologous protoplasm without vacuoles, implying that the nucleus is contained within them (Su and Huang, 1965).

Transmission

Transitory yellowing is transmitted by three species of *Nephotettix*, *N. apicalis* (Motschulsky) (Chiu et al., 1965), *N. cincticeps* (Uhler) (Chiu et al., 1968), and *N. impicticeps* Ishihara (Hsieh, Chiu, and Chen, 1970). It is not transmitted by *Cicadulina bipunctella*, *Nilaparvata lugens*, or *Recilia dorsalis* (Chiu et al., 1968). Attempts to transmit the virus by mechanical inoculation, by planting seeds (522 seeds tested) from infected rice plants of several varieties, and by growing young seedlings in soil taken from diseased field have failed (Chiu et al., 1965).

The virus not only persists in the vectors but it also multiplies in the vectors: the virus has been passed from insect to insect for seven serial transfers by microinjection technique. If there is no virus multiplication, the dilution is 10^{-20}, far beyond the dilution end point of the virus. Furthermore, the incubation period of injected insects becomes longer when a high dilution of virus inoculum is used for injection (Hsieh, 1969). The proportion of active transmitters of *N. apicalis* is 41 to 65 percent, of *N. cincticeps*, 35 to 71 percent (Chiu et al., 1968; Chiu and Jean, 1969), and of *N. impicticeps*, 47 percent (Hsieh et al., 1970). The shortest acquisition feeding period is 5 minutes for *N. apicalis* (Chiu et al., 1968), and 15 minutes for *N. cincticeps* (Chiu and Jean, 1969). The incubation period of the virus in *N. apicalis* is 8 to 34 days, mostly 9 to 16 days, in *N. cincticeps*, 21 to 34 days (Chiu et al., 1968), and in *N. impicticeps*, 4 to 20 days, mostly 10 to 12 days (Hsieh et al., 1970). However, Hsieh (1969) pointed out that the incubation period in *N. apicalis* varies according to the age of the insect, length of acquisition feeding period, and temperature. The incubation period gradually increases as the age of the insect at the time of acquisition feeding increases. When the insects have an acquisition feeding period of

less than 1 hour, the incubation period is longer. The insects do not become infective when the temperature is below 16 C or above 38 C. However, from 20 to 36 C, the higher the temperature, the shorter the incubation period. Transstadial passage exists. The virus persists in the vectors and the vectors often retain infectivity for their entire lives. The longest retention period is 55 days for *N. apicalis*. The daily transmission pattern is intermittent. There is no transovarial passage. The shortest inoculation feeding period is 5 to 10 minutes for *N. apicalis*. The incubation period in plant is 10 to 11 days (Chiu et al., 1968).

The virus

In the dip and clarified sap preparations of diseased leaves, the particles of transitory yellowing virus are bullet-shaped, 90 nm in diameter, and 120 to 140 nm in length, surrounded by two membranes with numerous outer projections. In ultrathin sections of diseased leaves, the bullet-shaped particles are approximately 94 nm in diameter and 180 to 200 nm in length. They are frequently arranged at the periphery of abnormal nuclei which are manifested by reduced chromatin substance and by the fine, uniform appearance of the nucleoplasm. During the early stage of infection, the particles are always limited to the phloem cells of diseased leaves (Chen and Shikata, 1968; Shikata and Chen, 1969).

The virus has been extracted from diseased leaves. The procedure is that 1 g diseased leaves, after being cut into small pieces, are homogenized in 10 ml of cold 0.1 M phosphate buffer, pH 6.8. The sap is centrifuged at 5,000 rpm for 30 minutes. The supernatant fluid is a virus preparation. The preparation can be further purified by differential centrifugation, and resuspending the pellet in phosphate buffer (Hsieh and Roan, 1967). The infectivity of the preparation is tested by injecting the preparation into virus-free *N. cincticeps*. The infectivity of the injected insects is determined by inoculation tests (Hsieh, 1967). The incubation period is related to the concentration of virus injected into the insects (Hsieh, 1969).

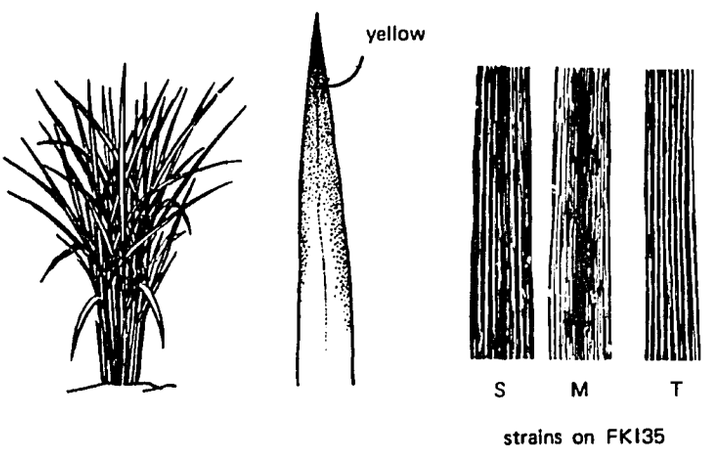
The virus preparation remains infectious at 0 to 2 C for 11 days but not for 12 days, and at 28 to 33 C for 36 hours but not for 48 hours. The thermal inactivation point is between 55.5 and 57.5 C for 10 minutes. The dilution end point is between 10^{-5} and 10^{-6} (Hsieh, 1967).

Host range

Echinochloa crusgalli and *E. colonum* are not hosts of the transitory yellowing virus (Chiu et al., 1968).

Varietal resistance

Miu (1964) reported that Kaoh-yu 10 was resistant to transitory yellowing. Based on field experiments with 55 rice varieties and lines, five varieties have been found to be highly resistant to transitory yellowing: Chu-tze, Chung-lin-chung, Hu-lu-tuen, Kaohsiung 22, and Wu-ku-chin-yu (Chiu, 1964). In 1965 and 1966, Hsieh et al. (1968) tested 49 varieties and found that Ching-kuo-gen, Ming-taug, Pai-ko-ching-yo, Shen-lo, and Wu-ko-ching-yo were resistant.



Symptoms of tungro disease.

TUNGRO DISEASE

(penyakit merah, yellow-orange leaf, and leaf yellowing)

"Tungro" in Ilocano (a Philippine language) means degenerated growth. It is generally considered that the disease was identified by Rivera and Ou (1965). It may have occurred in the Philippines for many years, however. Virus diseases had previously been reported in the Philippines: "stunt" or "dwarf" transmitted by "*Nephotettix bipunctatus* Fabr." (Agati, Sison, and Abalos, 1941), "accep na pula" (Tagalog, means red disease) or stunt transmitted by "*N. bipunctatus cincticeps* Uhler" (Serrano, 1957), "dwarf" transmitted by "*N. apicalis* var. *cincticeps* Uhl." (Reyes, 1957), "dwarf or stunt" transmitted by "*N. apicalis* var. *cincticeps* Uhl." (Reyes, Legaspi, and Morales, 1959), "tungro" or "dwarf" (Fajardo et al., 1962), and "tungro" transmitted by "*N. apicalis* (Motsch.)" (Fajardo et al., 1964). Except for Fajardo et al. (1964) the investigators emphasized the similarities of the diseases to rice dwarf reported in Japan.

These diseases are not identical with dwarf in Japan for the following reasons: 1) Differences in symptomatology such as yellowing of leaves, degree of stunting, and number of tillers of the infected plants particularly those inoculated artificially. 2) Unidentical species of insect vector, although the taxonomy of species of *Nephotettix* was quite confused before 1964. *N. cincticeps* is not known to exist in the Philippines, and *N. impicticeps* has not been reported to be a vector of dwarf in Japan. 3) Dissimilarities of virus-vector interaction.

For instance, no one had pointed out a definite incubation period of the virus in the vector. Successful transmission was often obtained by providing the insects an acquisition and inoculation feeding period of a few days. That was obviously shorter than the incubation period reported in Japan for the dwarf virus in its vector. Nor had anyone in the Philippines reported transovarial passage or transstadial passage although most of them cited Fukushi's paper (1934). No one had made an extensive study of serial transmission except Fajardo et al. (1964). The results obtained by Fajardo et al. indicated the loss of infectivity of the vectors because three out of five infective insects lost infectivity on the second and third transfers (only three transfers were made in the experiment). This result was quite different from the virus-vector interaction of dwarf. It was also the first indication of nonpersistence of a virus in its leafhopper vector. Before 1965, it was generally believed that all leafhopper-borne viruses were persistent in the vectors. This concept was also shared by Rivera and Ou in their paper (1965).

Actually, based on present knowledge, the Philippine rice virus diseases mentioned above are similar to tungro. If this conclusion is correct, tungro has been known in the Philippines at least since 1940 (Agati et al., 1941). Excluding Japanese workers, Agati et al. were the first to provide evidence of transmission of a rice virus disease.

"Penyakit merah" is Malay and means red disease. According to Singh (1969a) the first mention of penyakit merah appears to have been made by Coleman-Doscas in 1934. The viral nature of the disease was demonstrated by transmission with *N. impicticeps* by Ou et al. (1965). But, as pointed out by Singh (1969b), in the past the term penyakit merah has been applied to all sorts of disorders whose cause was not definitely established and to damage caused by leafhoppers, stemborers, rats, crabs, nematodes, iron toxicity, acidic soil conditions, drought, etc., as long as the rice plants turned orange or a shade of yellow or red. Consequently, Singh (1969b) proposed that wherever positive proof of the viral nature of the disease is present, "penyakit merah virus disease" should be used to specify it. Nevertheless, while the viral nature of penyakit merah has been confirmed by several investigators (Singh, 1969c; Lim, 1969; Ting and Paramsothy, 1970), no investigator has pointed out any difference between penyakit merah and tungro regardless of symptomatology, species of vector, virus-vector interaction, or varietal reaction. On the contrary, they concluded either that penyakit merah appears to be related to tungro (Ou et al., 1965; Singh, 1969c) or that penyakit merah and tungro are caused by the same virus (Ting and Paramsothy, 1970). But so far, no attempt has

been made to change the name of the disease from penyakit merah to tungro to reduce the confusion about names of rice virus diseases.

Yellow-orange leaf, according to Wathanakul and Weerapat (1969), was first observed in central Thailand in 1964, and later identified as a distinct virus disease by Wathanakul in 1965. However, Lamey, Surin, and Leeuwangh (1967) concluded that the yellow-orange leaf virus and the tungro virus are the same as revealed by similarities in symptomatology, species of vector, acquisition feeding period, incubation period (if any), inoculation feeding period, virus retention period, and varietal reaction. Furthermore, Saito et al. (1970) reported that the virus particles of yellow-orange leaf having a diameter of 30 nm are similar to those of tungro. "Yellow-orange leaf" still appears in recent literature instead of tungro (Saito et al., 1970; Wathanakul, 1969; Wathanakul, Chaimangkol, and Kanjanasoon, 1968).

Leaf yellowing in India was first reported by Raychaudhuri, Mishra, and Ghosh (1967a). Later, John (1968) demonstrated the presence of tungro in India and pointed out that the material used for his study was similar to that used by Raychaudhuri et al. (1967a). The term "leaf-yellowing" is gradually being replaced by tungro because recent publications (Govindu, Harris, and Yaraguntaiah, 1968; John, 1970; Mukhopadhyay and Chowdhury, 1970; Raychoudhuri and John, 1970) used tungro instead of leaf yellowing for the disease in India.

In addition to the distribution of tungro and similar diseases in the Philippines, Malaysia, Thailand, and India, tungro has also been identified in Indonesia (Rivera, Ou, and Tantere, 1968) and East Pakistan (Nuque and Miah, 1969; Gálvez-E. and Miah, 1969; Lippold et al., 1970). In general the disease is widely distributed in these countries. For instance, Lamey et al. (1967) made a survey of yellow-orange leaf in Thailand and concluded that about 660,000 hectares or about 10 percent of the rice area of Thailand was moderately or severely infected in 1966.

The yield loss due to tungro is determined by susceptibility of rice variety and plant age at the time of infection (Ling and Palomar, 1966; Ling, 1969d). For instance, in greenhouse experiments when IR8 plants are inoculated at 15, 30, 45, 60, and 75 days after sowing the yield reduction is 68, 57, 30, 16, and 7 percent, respectively (IRRI, 1967a). In contrast, for IR9-60, an experimental selection, the yield reduction is 74, 55, 50, and 32 percent when inoculated at 15, 30, 45, and 60 days after sowing, respectively. The yield is not significantly reduced when plants are inoculated at 90 days (Ling and Palomar, 1966).

Infected plants take longer to flower (Ling and Palomar, 1966). For instance, if IR8 is infected at 15 days, the delay in flowering is about 4 weeks. Thus, when most of the crop is ready for harvest, the grains of the infected plants are still immature. From a practical viewpoint, this factor also causes a considerable reduction in yield. In the field, a cage experiment showed that the yield reduction of seedlings of variety Seraup 50 inoculated with penyakit merah is 69 percent (Ou and Goh, 1966).

Symptoms

Tungro-infected rice plants, especially susceptible varieties, are stunted and the number of tillers is lightly reduced. The leaves are yellow, slightly rolled outward and somewhat spirally twisted. The plant becomes stunted through a shortening of both the leaf sheath and leaf blade. Because of the limited elongation of the new leaf sheath, the unfolded leaf is sometimes clasped by the outer leaf sheath. The degree of stunting varies among rice varieties and reduction in plant height decreases with increasing plant age at the time of infection. When IR9-60 is inoculated at 15, 30, 45, and 60 days after sowing, the reduction in plant heights is 52, 35, 15, and 4 percent, respectively (Ling and Palomar, 1966). Tillering is also influenced by the age of plant at the time of infection. The number of tillers is significantly reduced when plants are infected at the early stages of growth. The number may increase if infection occurs when the plant is more than a month old, but remains the same if infection takes place during the late growth stages. Yellowing, which ranges from light yellow to orange-yellow or brownish-yellow, usually starts from the tip of the lower leaves. The color varies among rice varieties and with environmental conditions. Irregularly shaped, dark brown blotches often develop on yellow leaves and occasionally on the green leaves, especially in infected young seedlings. The young leaves of infected plants are often mottled or have pale green to whitish stripes of various length running parallel to the veins. Root development is poor. Infected plants may die but usually they live until maturity. Infected plants take longer to mature because of delayed flowering. The panicles are often small, sterile, and not completely exserted. The grains are often covered with dark brown blotches and are lighter than those of healthy plants, but low yields mainly result from fewer grains per plant. Grain quality is not consistently or strikingly different from grain quality of healthy plants in percentage of milled rice, protein content, amylose content, or gelatinization temperature.

On some varieties, the symptoms of infection may be completely masked after a certain growth period. Later, the plants may again

show the symptoms, may develop symptoms only on the tillers, or may remain without symptoms.

The percentage of infection usually decreases with increasing plant age at the time of inoculation. For instance, when IR9-60 seedlings are inoculated at 15, 30, 45, 60, and 90 days after sowing, 93, 91, 68, 47, and 0 percent of the plants, respectively are diseased. Plants infected at late stages of growth may not develop symptoms before harvest but they may develop symptoms on regenerated growth when ratooned (Ling and Palomar, 1966).

Microscopic examination of sections stained with Giemsa solution revealed that some parenchyma cells in the vascular bundles of the diseased leaves contain stained, somewhat round inclusion bodies. The size of the inclusion bodies seems to vary with the size of the cell (IRRI, 1967a).

The leaf blades of diseased plants often become dark after being treated with iodine solution especially when the chlorophyll is removed by boiling the leaves in alcohol before staining. The reaction indicates the presence of starch in the leaf blades. This contrasts strikingly with the absence of the dark color reaction by the leaf blades of healthy plants. Possibly the virus affects carbohydrate metabolism by increasing starch synthesis, decreasing starch hydrolysis, or both. The starch reaction may be used to help diagnose the disease (IRRI, 1967a).

Transmission

No evidence has been found to indicate transmission of tungro through the seed in tests with 9,000 seeds collected from infected plants (IRRI, 1967a; Singh, 1969c), or through soil, or consistently by mechanical means.

The virus, however, is known to be transmitted by *Nephotettix impicticeps* Ishihara in India (*tungro:* John, 1968; Mukhopadhyay and Chowdhury, 1970; *leaf yellowing:* Raychaudhuri et al., 1967a), in Indonesia (*tungro:* Rivera et al., 1968), in Malaysia (*penyakit merah:* Ou et al., 1965; Singh, 1969a, 1969c; Lim, 1969; Ting and Paramsothy, 1970), in East Pakistan (*tungro:* Nuque and Miah, 1969; Gálvez-E. and Miah, 1969), in the Philippines (*tungro:* Rivera and Ou, 1965; Ling, 1966), and in Thailand (*tungro:* Lamey, Surin, and Leeuwangh, 1967; *yellow-orange leaf:* Wathanakul and Weerapat, 1969). It is also transmitted by *N. apicalis* (Motschulsky) (*tungro:* Fajardo et al., 1964; Rivera and Ling, 1968; Gálvez-E. and Miah, 1969; Ling, 1970; *yellow-orange leaf:* Wathanakul et al., 1968), by the hybrids of *N. impicticeps* and *N. apicalis* (*tungro:* Ling, 1968c), and by *Recilia dorsalis* (Motschulsky) (*tungro:* Rivera et al., 1969; *yellow-orange leaf:* Wathanakul, 1969).

Negative transmission of the disease by *N. apicalis* has been obtained by various investigators (John, 1968; Lamey, Surin, and Leeuwangh, 1967; Ling, 1968c; Ou and Rivera, 1969, Singh, 1969a). *N. apicalis,* however, is less able to transmit the tungro virus than *N. impicticeps* because the percentage of active transmitters, virus retention period, and number of disease-transmitting days of *N. apicalis* are significantly lower than those of *N. impicticeps* (Ling, 1970). Probably, negative results are caused by the variability of the active transmitters among different insect collections. In other words, if the insects collected for the transmission study have a very low percentage of active transmitters it is difficult to obtain positive transmission unless many insects are used. Another possible reason is insufficient acquisition feeding. Since the transmissive efficiency of *N. apicalis* is low, the insects must be provided with a maximum amount of the virus by prolonging the acquisition feeding period. Extending this period to 4 or 5 days would increase the likelihood of positive transmission especially when the insects are tested daily and are allowed to have daily reacquisition feeding.

The virus does not persist in the vector (Ling, 1966). Similar results have been obtained by various investigators (Gálvez-E. and Miah, 1969; John, 1968; Lamey, Surin, and Leeuwangh, 1967; Lim, 1969; Nuque and Miah, 1969; Rivera and Ou, 1967; Rivera et al., 1968; Singh, 1969c; Ting and Paramsothy, 1970; Wathanakul et al., 1968). The percentage of active transmitters varies among methods of testing. The proportion of active transmitters of *N. impicticeps* ranges from an average of 35 percent (Singh, 1969a) to 83 percent (Rivera and Ou, 1965). In a small sample, however, it is not uncommon to find that every insect tested transmits the disease. Zero to twenty-seven percent of *N. apicalis* are transmitters (Ling, 1970), 15 percent of the hybrids are, and 4 percent (IRRI, 1968) to 8 percent (Rivera et al., 1969) of *R. dorsalis* are.

The shortest acquisition feeding for *N. impicticeps* is 5 minutes (Singh, 1969c) to 30 minutes (Rivera and Ou, 1965; John, 1968; Lim, 1969). There is no demonstrable incubation period, but if it exists, it cannot be longer than 2 hours because virus-free *N. impicticeps* can transmit the disease by having acquisition and inoculation feeding periods of 1 hour each (Ling, 1966). Rivera and Ou (1965) stated that a 24-hour incubation period appeared to be necessary for the adult insect to transmit the disease because the shortest interval they tested was 24 hours. Infective insects usually transmit the disease immediately after acquisition feeding and every day until they lose their infectivity. Once they lose their infectivity they remain noninfective for the rest of their lives unless given an access to another virus source. Hence, the daily transmission

pattern is consecutive but the hourly transmission pattern is intermittent (Ling, 1966; 1969a). The insects gradually lose their infectivity with time even in hours (Ling, 1966; Singh, 1969c). More than 50 percent of the infective insects become noninfective 24 hours after the termination of acquisition feeding. The longest retention period is 6 days (Wathanakul and Weerapat, 1969). The longest retention periods obtained by others are 2 days (John, 1968), 3 days (Lim, 1969), 4 days (Rivera and Ou, 1967; Singh, 1969c), and 5 days (Ling, 1966; Ting and Paramsothy, 1970). The longest retention period is 3 days for *N. apicalis* (Ling, 1970), and 4 days for *R. dorsalis* (Rivera et al., 1969).

There is no transovarial passage, nor transstadial passage. Hence, the infective nymphs of *N. impicticeps* lose their infectivity after molting (Ling, 1966). An explanation for the loss of infectivity after molting is that the virus particles are distributed only on the surface of the stylets and on the lining of the alimentary canal. When the insect molts, the virus particles are cast off together with the stylets and general cuticles. This interpretation is supported by the facts that old stylets are present in the exuviae (cast skin) and that new stylets form inside the old ones (IRRI, 1971). Insects without virus particles cannot be infective. Obviously when the insects become recontaminated with virus after molting they should become re-infective. That is why after molting the insects can regain infectivity after a reacquisition feeding.

The length of acquisition feeding period affects the percentage of infective insects (Rivera and Ou, 1965; Ling, 1966; Ting and Paramsothy, 1970) and the retention period (Ling, 1966; Ting and Paramsothy, 1970). An acquisition feeding period of 3 to 5 days often provides the insects with a maximum amount of the virus. The shortest inoculation feeding period is 7 minutes (Ling, 1968b). The shortest inoculation feeding period obtained by others are 10 minutes (Singh, 1969c), 15 minutes (Rivera and Ou, 1965; John, 1968), 20 minutes (Wathanakul and Weerapat, 1969), and 30 minutes (Lim, 1969). A single probing by an infective insect can cause a seedling to become infected (Ling, 1968b). The incubation period in the plant varies from 6 days (Rivera and Ou, 1965) to 15 days (Wathanakul and Weerapat, 1969).

The transmissive ability of both *N. impicticeps* and *N. apicalis* is not correlated to morphological features of the insects such as tegminal spot, length of mandible, maxilla, and aedeagus, and number of teeth on aedeagus (IRRI, 1968; Ling, 1970).

The virus seems to have no deleterious effect on *N. impicticeps* because there are no significant differences in life span, fecundity,

and egg hatching rate between viruliferous and virus-free insects (IRRI, 1967b; Ling, 1968d).

The infectivity of viruliferous *N. impicticeps* can be inactivated by formalin (Ling, 1968d), other chemicals, and low pH (IRRI, 1971).

The virus

The first electron micrograph of tungro virus particles in an ultrathin section of diseased leaf was made by Dr. E. Shikata in early 1967. Shikata's result has been published only by Ou and Ling (1967) who adopted 30 to 35 nm as the size of particles. Later, Gálvez (1968a) purified the virus and reported that the particles are polyhedral and 30 to 33 nm in diameter. Recently, Saito et al. (1970) reported that the virus particles of yellow-orange leaf are polyhedral with a diameter of 30 nm.

The purification procedure used by Gálvez (1968a) is as follows: Diseased leaves are homogenized with three volumes of 0.01 M EDTA at pH 6.8 and squeezed through cheesecloth. The filtrate is then heated at 40 C for 1 hour and centrifuged at 7,000 *g* for 20 minutes. The supernatant liquid is centrifuged at 100,000 *g* for 30 minutes. The pellet is suspended in 0.01 M EDTA and centrifuged at 7,000 *g* for 20 minutes. The supernatant liquid is then subjected to density-gradient centrifugation at 24,000 rpm for 3 hours in the SW 25.1 Spinco rotor. The gradients are prepared by placing, in order, 4, 7, 7, and 7 ml of 100, 200, 300, and 400 mg sucrose per ml of EDTA buffer in a tube. The visible zone, located 3.0 cm below the meniscus, is collected and dialyzed against water for 24 hours or centrifuged at 100,000 *g* for 30 minutes. The pellet is suspended in EDTA. The virus preparation is obtained after the suspension is centrifuged at 7,000 *g* for 15 minutes.

Saito et al. (1970) used a different purification procedure: Diseased leaves are homogenized with three volumes of 0.5 M citrate buffer at pH 6.5 and 0.001 M EDTA, and squeezed through cheesecloth. After adding carbon tetrachloride (20%), the filtrate is stirred for 15 minutes and centrifuged at 10,000 rpm for 15 minutes. The water layer is then mixed well with polyethylene glycol 6000 (8%), and centrifuged at 10,000 rpm for 15 minutes. The pellet is suspended in 0.005 M borate buffer at pH 9.0 and 0.001 M EDTA and centrifuged at 10,000 for 15 minutes. After repeating the last two steps three times, the supernatant fluid is centrifuged at 30,000 rpm for 1 hour. The pellet is suspended in 0.005 M borate buffer at pH 9.0 and 0.001 M EDTA. The purified virus preparation is obtained after the suspension is centrifuged at 10,000 rpm for 15 minutes.

According to Gálvez (1968a), the physicochemical characteristics of the virus determined by analytical density-gradient centrifugation indicated that the virus withstands temperatures below 63 C for 10 minutes and pH values up to 9, without apparent denaturation. Nor is the virus denatured *in vitro* for more than 24 hours at room temperature. The sedimentation coefficient is 175 ± 5S.

The virus has different strains. Rivera and Ou (1967) reported the "S" and "M" strains of the virus. The former is more widely distributed in the Philippines. Although symptoms produced by these two strains are similar in most rice varieties such as IR8, Milfor 6(2), Palawan, Taichung Native 1, and Tainan 3, they can be differentiated in such varieties as Acheh, FK 135, and Pacita. The symptoms produced by the "S" strain on these three varieties is conspicuous interveinal chlorosis, giving an appearance of yellow stripes and sometimes irregular chlorotic specks on young leaves. On the other hand, the "M" strain produces mottling. The growth retardation of FK 135 caused by the "S" strain is much greater than that caused by the "M" strain.

Recently, C.T. Rivera *(personal communication)* obtained another strain from La Trinidad, Benguet, Philippines. It was designated as the "T" strain. The new strain incites narrow leaf blade on Taichung Native 1, IR5, IR8, and IR22 but produces interveinal stripes on FK 135 which closely resemble the stripes caused by the "S" strain. The "T" strain, however, retards growth much less than does the "S" strain, even less than does the "M" strain. So far the susceptibility of rice varieties to these three strains in terms of percentage of infected seedlings seems to be similar.

In India, Raychoudhuri and John (1970) reported that a recent outbreak of yellowing in northern India revealed that a more virulent strain of tungro (RTV_2) is involved. But they pointed out also some varieties, most of which are local *indicas*, are resistant to both RTV_1 and RTV_2.

The antiserum obtained by injecting rabbits with partially purified virus preparation also reacts with other viruses in the Philippines (John, 1965).

Host range

In India, Raychaudhuri et al. (1967a) reported that in addition to leaf yellowing, chlorosis has been observed on some grasses in paddy fields at Cuttack such as *Leersia hexandra*, *Rottboellia compressa*, *Cynodon dactylon*, and two unidentified grasses.

In the Philippines, Wathanakul (1964) inoculated 29 species of plants with viruliferous insects and found that *Eleusine indica*, *Echinochloa colonum*, and *E. crusgalli* are also the host plants of the

tungro virus. The symptoms of infected *E. indica* are yellowish white specks along the veins and gradual drying up of the leaf tip. The infected plants produce many tillers. Some of the infected tillers eventually die. The symptoms of the naturally infected *E. indica* are stunting of the younger tillers, partial yellowing of lower leaves (ranging from yellow to pale orange discoloration starting from the tip), and yellowish white specks along the veins. No symptoms are observed on *E. colonum* and *E. crusgalli*. By using the vector, the virus was, however, recovered from inoculated plants of these three species, and transmitted to rice seedlings later. Rivera, Ling, and Ou (1969) reported that among 63 species of graminaceous plants inoculated, the following species were infected (all had less than 6 percent infection except species of *Oryza* which showed more than 50 percent infection):

Dactyloctenium aegyptium
Eragrostis tenella
Ischaemum rogosum
Leersia hexandra
O. barthii
O. officinalis
O. ridleyi
O. rufipogon
Paspalum scrobiculatum
Setaria glauca
Sorghum vulgare
Triticum aestivum

The infected plants develop symptoms similar to those of tungro in rice. But it was difficult to recover the virus from the infected plants and transmit it to rice. The infection of *E. colonum* could not be confirmed.

Varietal resistance

The first field test for varietal resistance to tungro was made by Fajardo et al. (1964) in 1962. They considered a loss from the disease of a third or more of the crop to indicate a susceptible variety, while they regarded slight or no infection as indicating resistance to the disease. They concluded that Balao, BPI-76, Camoros, Kao Bai Sri, Macabio, Macatampal, Mancasar, Raminad, Red Tagetep, Wagwag and other local Philippine varieties are susceptible, while BE 3 and Peta were resistant. Later, 66 varieties were field-tested for resistance to tungro at IRRI (IRRI, 1964). The most extensive field test for resistance to yellow-orange leaf was initiated at the Bangkhen Rice Experiment Station in 1965 (Thailand Ministry of Agriculture, 1966). From 1965 to 1968, 7,126 varieties and selections were tested at the Station, and 1,661 of them are listed as resistant (Wathanakul, 1969).

Information about testing varieties for tungro resistance by artificial inoculation in greenhouse first became available at IRRI in 1963. The technique was improved, and the mylar cage method

was developed (IRRI, 1964). This method is widely used for a small scale testing. For large scale testing, a mass screening method was developed in 1965 (Ling, 1967, 1969c). The method permits about a thousand seedlings a day to be inoculated. From 1965 to 1969, in addition to rice varieties, genetical materials, and duplicates, 9,718 IRRI selections consisting of more than 300,000 seedlings were tested by the mass screening method at IRRI (Ling and Aguiero, 1970).

Based on the results of artificial inoculation, the following varieties are resistant in different countries.

India—Tungro (Raychoudhuri and John, 1970).

8622	Latisail
Ambemohar 159	NC 1626
Bhadas 1303	NSJ-198
Intan	Pankhari 203
IR20	Sigadis
Kamod	T-47 (Faizabad)
Kataribhog	Tilakchari

Indonesia—Tungro (Rivera et al., 1968)

Dara	Peta
Pankhari 203	Snytha

Malaysia—Penyakit merah (Ou et al., 1965; Singh, 1969c)

Bengawan	Peta
FB 24	Sigadis
Gam Pai	Tjeremas
Intan	Tjina
Latisail	Tjina 417
Pankhari 203	

Philippines—Tungro (IRRI, 1967b, 1968; Ling, 1969c)

59-33 ! (B11 x Mas)	Adday sel.
221/BCII/11/3	Andifrom N. Pokhara
221/BCIIIA/81 ? 1	Badshahbog T412
221/BCIV/1/45/10	Basmati 37
221/BCIV/1/178/11	Basmati 370
221b/57/1/4	Bengawan
221b/210/2/1/1/2	C18
221b/212/2/2/2/1	Chung Ta 312 Hao x Binastian
221b/236/2/3/2/1	DV 29
221c/53/1/2/1	Fadjar
221c/53/1/3/1	FB 24
221c/291/1/3/3	Gam Pai 30-12-15
221e/20/3	H 4
268/Pr/2/2/2	HR 21
6517	Indrasail
Adday local sel.	JC-170

Kai Lianh Hsung Tieng
Ladang
Lang Chung Yi Lung Ju
Lantijang
Latisail
M. Sungsong
Mas
Padi Kasalle
Pankhari 203
Pehkohak-Kimkan
Peta
PI 160677-2
PI 160677-4
PI 160677-5
PI 184675-2
PI 184676
Pien Chan Ying Tao
Podiwi A8
Rajamandal Baran
Ram Tulasi
Red rice
Salak 2885
Seratus Hari T/36
Seri Raja
Sigadis
Tilakkachray
Tjahaja
Tjeremas
Tsou-yuen
Urang-Urangan 89
Yi Shih Hsing

Thailand—Yellow-orange leaf (Wathanakul and Weerapat, 1969)

221/BCIIIA/81/2/1
221c/20/3
221c/391/1/3/3
Bengawan
H 4
Latisail
Pankhari 203
Peta
Sigadis
Tjeremas

By the mass screening method, 162 duplicated entries of Pankhari 203, consisting of 4,462 seedlings were tested. From 0 to 21 percent of the seedlings of individual entries were infected with an average of 4.6 percent whereas an average of 98.8 percent of the seedlings of the susceptible check, Taichung Native 1, were infected (Ling, 1968b). Thus Pankhari 203 is one of the most resistant varieties. Tests of most investigators in different countries generally agree with this finding: India (John, 1968; Raychoudhuri and John, 1970), Indonesia (Rivera et al., 1968), Malaysia (Singh, 1969c), Philippines (Rivera and Ou, 1967), and Thailand (Wathanakul and Weerapat, 1969). In East Pakistan, however, Gálvez-E. and Miah (1969) reported 30 percent or more infection of this variety. The reason is not clear but their results (Table 2 of their paper) indicated that seedlings of Pankhari 203 had the lowest percentage of infection among the 10 varieties they tested by mass inoculation.

Pankhari 203 is not only resistant to tungro but also resistant to the vector, *N. impicticeps* (Ling, 1968b). The resistance of Pankhari 203 to *N. impicticeps* has been confirmed by entomologists (Pathak, Cheng, and Fortuno, 1969). This leads to the suspicion that resistance to tungro is associated with resistance to the vector. Excluding intermediate types, however, only four theoretical combinations of resistance to the virus and to the vector are possible: 1) resistant to the virus, resistant to the vector, 2) resistant to the virus, suscepti-

ble to the vector, 3) susceptible to the virus, resistant to the vector, and 4) susceptible to the virus, susceptible to the vector. Pankhari 203, Kai Lianh Hsung Tieng, IR8, and Taichung Native 1 can, respectively, serve as examples for these four combinations (Ling, 1969c). Furthermore, IR8 is more resistant to the insect vector, but more susceptible to the tungro virus than Pehkohak-Kimkan (IRRI, 1970). Consequently, resistance to the tungro virus may not always be correlated with resistance to the insect vector, *N. impicticeps*.

The mechanism of tungro resistance in Pankhari 203 is not due to the inability of *N. impicticeps* to feed on it although sclerenchyma caps are present on the abaxial of the vascular bundles in the cross-section of the leaf sheath. The feeding behavior of the insect on Pankhari 203 and on Taichung Native 1 is similar; the size, distribution, and number of feeding punctures and of feeding tracks terminating at vascular bundle do not differ markedly. Since the percentage of infective insects declines after the insects feed on Pankhari 203 seedlings, tungro-resistance in Pankhari 203 may be caused by the inactivation of the virus or by inhibition of virus multiplication by a substance or substances present in the plant (Ling, 1968c).

Pankhari 203 has been used as a resistant donor parent in the breeding program by breeders at the IRRI; 2,448 selections from the crosses with Pankhari 203 have been tested, 19 percent of them are in resistant group (Ling and Aguiero, 1970).

YELLOW DWARF DISEASE

Yellow dwarf is a translation of Japanese "ooi-byo." In Malaysia, the disease was first called "padi jantan" (Lim and Goh, 1968) which is Malay and means male paddy. This name of the disease has been followed by Singh, Saito, and Nasu (1970). However, Lim (1970) proposed changing "padi jantan" to yellow dwarf, because of the similarities of these two diseases.

According to Hashioka (1964), the yellow dwarf first appeared in literature in the annual report of Kochi Agricultural Experiment Station in 1919. In Taiwan, the disease has been known since before 1932 (Kurosawa, 1940). Kurosawa was the first to suspect the viral nature of the disease and the transmission of the disease by rice leafhoppers for reasons such as symptomatology, variation of disease incidence in the field, and a higher disease incidence when artificial light was provided during the night. According to Shinkai (1962), *Nephotettix cincticeps* was suspected as a vector of the disease in 1943 when it was described in the report of Kochi Agricultural Experiment Station, and in 1948, Enjoji re-examined the possibility and considered the insect as a vector (Shinkai, 1962). It was Iida and Shinkai (1950), however, who first proved by experiments that *N. cincticeps* is a vector of the disease.

In addition to Japan, Taiwan, and Malaysia, the disease is widely distributed in Asia: Ceylon (Abeygunawardena, Bandaranayaka, and Karandawela, 1970), India (Raychaudhuri, Mishra, and Ghosh, 1967a, 1967b; Pathak et al., 1967; Govindu, Harris, and Yaraguntaiah, 1968; Raychoudhuri and John, 1970), Okinawa (Shinkai, Miyanaga, and Tobechi, 1963), East Pakistan (Gálvez E. and Shikata, 1969), the Philippines (IRRI, 1963; Palomar and Rivera, 1967), southern China (Hashioka, 1952), and Thailand (Wathanakul and Weerapat, 1969).

Symptoms of yellow dwarf disease.

In tropical regions, yellow dwarf usually causes little yield loss because it occurs only sporadically and the infection often takes place during the late stages of plant growth. A high incidence of late infection, as shown by severe symptoms on regenerated (ratoon) growth after harvest, is not uncommon. In Taiwan, the disease causes great losses particularly in the second crop of the year. Hashioka (1964) reported a massive infection in Kochi, Japan, in which 70 to 80 percent of the hills in the field were infected, resulting in a yield loss of 50 percent.

The yield loss of individual plants is determined by plant age at the time of infection. In Japan, when rice seedlings are inoculated at the 11-leaf stage or earlier, the plants produce no grains. When inoculated at the 12- and the 13-leaf stage, the yield reduction is 79 and 22 percent, respectively. When inoculated at the 14-leaf stage or later, the yield reduction is not significant (Shinkai, 1962). Similarly, in the Philippines, BPI-76 inoculated 10 or 30 days after sowing fails to yield fertile grains, but there is no significant yield reduction when inoculated at 60 days (Palomar and Rivera, 1967).

Symptoms

The first symptoms of yellow dwarf is general chlorosis, especially on the newly emerged and young leaves. The color varies from yellowish-green to whitish-green. As the disease progresses, the infected plants become severely stunted, tillering increases markedly, and leaves become soft and droop slightly. The infected plants may die but they often remain alive until maturity. The infected plants produce either no panicles or a few, small panicles bearing mostly unfilled grains.

Plants infected during the later growth stages may not show the characteristic symptoms before harvest. The symptoms are conspicuous on the regenerated growth when the plants are ratooned, however.

The older the plant at the time of infection, the less severe the reduction in plant height is (Shinkai, 1962). When inoculated at 10 and 39 days after sowing, the plant height of BPI-76 is reduced 51 and 45 percent, respectively, but no appreciable reduction occurs when the variety is inoculated at 60 days (Palomar and Rivera, 1967).

Transmission

Yellow dwarf is transmitted by three species of *Nephotettix: N. cincticeps* (Uhler) (Iida and Shinkai, 1950), *N. impicticeps* Ishihara (Shinkai, 1959, 1962), and *N. apicalis* (Motschulsky) (IRRI, 1963;

Ouchi and Suenaga, 1963; Shinkai et al., 1963). Shinkai (1962) found that several insects did not transmit the disease in experiments: *Inemadara oryzae, Laodelphax striatellus, Macrosteles fascifrons, M. quadrimaculatus, Nilaparvata lugens, Nisia atrovenosa, Recilia dorsalis, Sogatella furcifera,* and *Tettigella viridis.* Later, Lim (1969) added two species, *Tettigoniella spectra* and *Scotinophara coarctata,* to the list. There is no evidence of the transmission of the disease through seeds although Shinkai (1951) and Lim (1970) have tested 4,983 seeds from infected plants. No positive results have been obtained by transmission through soil (Lim and Goh, 1968) or by mechanical means (Lim, 1970).

N. apicalis has been reported to transmit yellow dwarf in Ceylon (Abeygunawardena et al., 1970), Japan (Ouchi and Suenaga, 1963; Shinkai, 1965), Okinawa (Shinkai et al., 1963), the Philippines (Palomar and Rivera, 1967), and Taiwan (Chiu, 1964); *N. cincticeps* in Japan (Iida and Shinkai, 1950) and Taiwan (Chiu, 1964); and *N. impicticeps* in Ceylon (Abeygunawardena et al., 1970), India (Raychaudhuri et al., 1967b), Japan (Shinkai, 1959), and the Philippines (Palomar and Rivera, 1967).

The causal agent has been established to be persistent in the vectors. The percentage of active transmitters is generally high but it is often difficult to obtain a precise figure because of mortality of the insects during the incubation period of the causal agent in the vectors. Nevertheless, Palomar and Rivera (1967), reported 69% active transmitters for *N. apicalis,* Shinkai (1962) reported 88% to 96% for *N. cincticeps,* and Palomar and Rivera (1967) reported 83% for *N. impicticeps* while Shinkai reported 94%.

The shortest acquisition feeding period is 10 minutes for *N. cincticeps* (Shinkai, 1962) and 10 minutes (Lim, 1970) to 30 minutes (Shinkai, 1962; Palomar and Rivera, 1967) for *N. impicticeps.* The incubation period in the vectors is generally long. It is 20 to 35 days, mostly 22 to 27 days for *N. apicalis* (Palomar and Rivera, 1967); 26 to 40 days, average 32 days, for *N. cincticeps* (Shinkai, 1962); and 20 days (Palomar and Rivera, 1967; Raychaudhuri et al., 1967b; Lim, 1970; Abeygunawardena et al., 1970) to 55 days (Abeygunawardena et al., 1970) with an average of 34 days in Japan (Shinkai, 1962) and mostly 20 to 26 days in the Philippines (Palomar and Rivera, 1967) for *N. impicticeps.* Transstadial passage occurs. The infective insects are usually obtained by providing acquisition feeding to the insects at the nymphal stage because of the long incubation period. Once the insects become infective, they remain infective for the rest of their lives. The longest retention period obtained is 38 days for *N. apicalis* (Palomar and Rivera, 1967), 103 days for *N. cincticeps* (Shinkai, 1962), and 27 days (Lim, 1970)

to 104 days (Shinkai, 1962) for *N. impicticeps*. There is no evidence of transovarial passage. The shortest inoculation feeding period is 5 minutes for *N. cincticeps* (Shinkai, 1962) and 2 or 3 minutes (Palomar and Rivera, 1967; Shinkai, 1962) to 10 minutes (Lim, 1970) for *N. impicticeps*. The incubation period in plants varies from 23 days (Palomar and Rivera, 1967) to 90 days (Shinkai, 1962; Abeygunawardena et al., 1970).

Temperature affects the transmissive ability of the insects and the length of incubation period (Ishii, Yasuo, and Ono, 1969). For instance, when the temperature during inoculation feeding is 10, 15, and 20 C, the average incubation period is 35, 34, and 28 days, respectively. When the temperature during an acquisition feeding period of 4 hours is 5, 10, 15, 20, and 25 C, 0, 7, 27, 64, and 73 percent of the insects, respectively, are infective. When viruliferous *N. cincticeps* are incubated at 10, 15, 20, and 25 C, 60, 60, 100, and 100 percent of the insects, respectively, are infective and they have, respectively, 2.7, 4.2, 14.0, and 16.1 disease-transmitting days. When the temperature during inoculation feeding period is 10, 15, and 25 C, 4, 62, and 85 percent of the insects, respectively are infective. Hence, the transmissive ability of *N. cincticeps* declines as temperature decreases.

Cytohistological changes occur in *N. cincticeps* when it acquires the causal agent of yellow dwarf. Before the end of the incubation period, 10 to 15 days after acquisition feeding, nuclei of the fat body cells enlarge and become irregular in shape. After 20 days the enlarged nuclei seem to shrink, and their irregular shapes become more pronounced. At the same time, vacuolation of the cytoplasm becomes apparent. Finally, about 25 days after acquisition feeding, shrinkage of the nuclei reaches its maximum, and the vacuoles in the cytoplasm increase so greatly in number that the fatty body cell appears to be completely reticulated. There are also the cytochemical changes in the vector. Both Feulgen reaction and methyl green staining tend to increase in intensity at the beginning of the infection, presumably because of an increase in the DNA content. The cytoplasm stains heavily with pyronine, suggesting an increase in RNA content. With time, both reactions diminish and are weakest when the peak of the vacuolation is attained, that is, upon the completion of incubation of the causal agent in the vector (Takahashi and Sekiya, 1962).

The causal agent

The nature of the causal agent of yellow dwarf is not completely settled at present. Takahashi (1964) obtained a purified preparation from diseased leaves by differential centrifugation. The pre-

paration not only contains virus particles which are polyhedral and 55 nm in diameter but also is infectious when tested by injecting it into virus-free *N. cincticeps*. Fuyaka and Nasu (1964) observed virus particles in the fatty body of the vector 29 days after acquisition feeding.

But evidence has mounted that the causal agent of yellow dwarf is presumptive mycoplasma since Nasu et al. (1967) reported that mycoplasma-like bodies are present in midgut and salivary glands of viruliferous *N. cincticeps* and *N. apicalis* and also in phloem cells of diseased plant tissues in Japan. Mycoplasma-like bodies have been found in phloem cells of diseased materials from India (Sugiura et al., 1968), the Philippines (Shikata et al., 1968; Shikata, Maramorosch, and Ling, 1969), East Pakistan (Gálvez E. and Shikata, 1969), Malaysia (Singh, Saito, and Nasu, 1970), and Thailand (Saito et al., 1970), and again in midgut and salivary glands of viruliferous insects (Sugiura et al., 1968). The bodies have been observed in salivary glands 17 days after acquisition feeding (Sugiura et al., 1969). The bodies are polymorphic (spherical to oval or irregular), variable in size (ranging from 80 to 800 nm), devoid of cell walls, bounded by unit membranes, and appear dense with a granulated mass or a clear central area and a dense peripheral cytoplasmic region. No direct proof for the mycoplasmal etiology of yellow dwarf has been found so far. In other words, the mycoplasma has not been cultivated on media nor has the disease been reproduced experimentally by the culturing of the organism to fulfill Koch's postulate and establish mycoplasma as the causal organism of the disease.

The infectivity of the extracts of salivary glands and midgut of viruliferous insects has been tested by injecting the extracts into virus-free *N. cincticeps* at the nymphal stage. The insects become infective only when injected with the fresh extracts. Extracts stored at different low temperatures for various number of days have no effect (Sugiura et al., 1969).

Since mycoplasma is known to be sensitive to some antibiotic substances, attempts have been made to apply antibiotics to diseased plants to obtain circumstantial evidence of the nature of the causal agent. Sugiura et al. (1968) first pointed out that the development of symptoms is delayed when the seedlings are treated with compounds of the tetracycline group at 0 to 5 days after inoculation but treating diseased plants with the compounds has no effect.

Four antiobiotics, tetracycline hydrochloride (Achromycin), chlortetracycline (Aureomycin), dimethyl chlortetracycline (Ledermycin), and oxytetracycline (Terramycin), at concentrations from 10 to 1,000 ppm, have been applied as foliage spray or root dipping,

or both, to diseased plants or to seedlings before or after inoculation to study their effect on development of symptoms. When the antiobiotics are applied as foliage spray to diseased plants every other days for a period of 20 days, yellow leaves of some diseased plants seem to be a slightly greener and newly grown leaves tend to be symptomless. The recovery is temporary, however, because the symptoms sometimes reappear on the recovered leaves after the termination of spraying (Sakurai and Morinaka, 1970). Spraying the foliage three times immediately before or after inoculation does not suppress the symptom expression (Sakurai and Morinaka, 1970).

When the roots of diseased plants are dipped in antiobiotics for 2 days, remission of the symptoms occurs in some plants but the symptoms reappear by 48 days after the treatment (Sakurai and Morinaka, 1970). Singh et al. (1970) demonstrated that when diseased plants are immersed in 1,000 ppm solution of Aureomycin for half an hour and sprayed with the solution at 3-day intervals for 2 weeks plant height increases slightly but tiller number decreases markedly by 2 weeks later. Gálvez E. and Shikata (1969) reported that diseased plants apparently become normal if their roots are dipped in 100 ppm solution of Aureomycin before transplanting in pots.

According to Sugiura, Kaida, and Osawa (1969), the tetracycline compounds effectively suppress the symptoms only when applied as a root dip at a concentration of about 40 ppm, for 24 hours shortly before or after inoculation and especially when the compounds are applied to the seedlings at 5-day intervals. The inoculated plants may develop symptoms when the treatment is suspended, however. On the other hand, Sakurai and Morinaka (1970) reported that the symptoms are suppressed by dipping the roots in the antibiotic solution for 24 hours before inoculation or immediately after, 10 days after, or 20 days after inoculation.

The transmissive ability of *N. cincticeps* treated with tetracycline compounds 1 day after acquisition feeding decreases within 33 days (Sugiura, Kaida, and Osawa, 1969). Sakurai and Morinaka (1970) pointed out that the percentage of infective insects decreases when the insects feed on antibiotics at 50 ppm before or after acquisition feeding.

High temperature suppresses the symptom development of inoculated seedlings and the transmissive ability of viruliferous *N. cincticeps* (Takasaki, Sugiura, and Iida, 1970). When inoculated seedlings are subject to the treatment for 2 to 11 days, the percentage of seedlings showing symptoms decreases as the duration at 40 C increases. The incubation period of viruliferous insects increases in accordance with duration of treatment at 40 C.

Host range

In addition to rice, Shinkai (1951, 1962) inoculated 30 species of plants and found that *Alopecurus aequalis*, *Glyceria acutiflora*, and *Oryza cubensis* are also the hosts of the causal agent of yellow dwarf.

Varietal resistance

The first observation on varietal reaction to yellow dwarf in the field was made by Kurosawa (1940) in Taiwan in 1932. From 1946 to 1948, Hashioka (1952) made field tests in Taiwan on 311 rice varieties from different countries and found 151 that showed 0 to 6 percent infection. Also in Taiwan, Chiu (1964) reported that Nan-gai-yu 27 and Hwalien-yu showed the lowest percentage of infected plants among 46 varieties and selections tested in 1963. In Japan, Komori and Takano (1964) found that Kaladumai, Loktjan, Pe Bi Hun, Saitama-mochi No. 10, and Tetep were highly resistant in the field. Recently Morinaka and Sakurai (1969, 1970) reported that the following varieties, having less than 10 percent infected seedlings, were resistant in the field:

Bason Takakal
Belle Patna
Blue Bonnet
Chiang-nan-tao
Chiem Chank
Karalath (H33)
Keau N 525
Loktjan
Naozane-mochi
Pa-shih-tze-sien
Russia No. 25
Russia No. 33
Russia No. 35
Tao-ren-chiao
Tetep
Yang-sien-tao

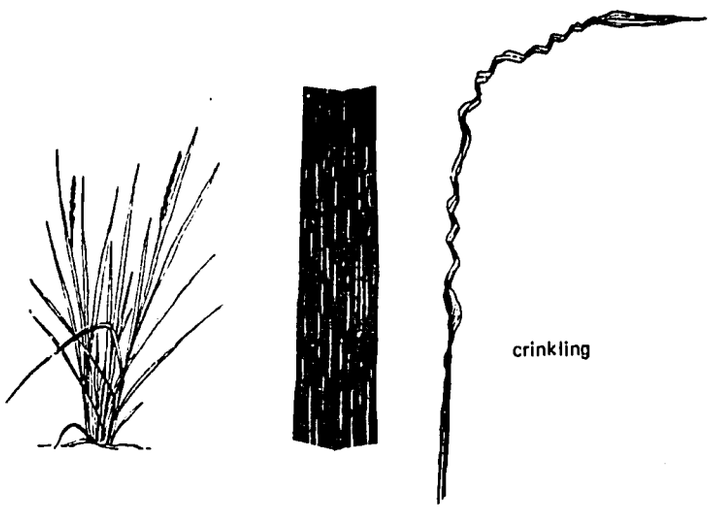
A method of testing rice seedlings for varietal reaction to yellow dwarf by artificial inoculation was developed in Japan (Sakurai, 1969; Morinaka and Sakurai, 1970). Since the causal agent has a long incubation period in both the insect vector and the rice plant, nymphs of *N. cincticeps* at first or second instar are confined on diseased plants for 2 to 3 days. They are used for inoculating seedlings 30 days after acquisition feeding when they have become adults and the incubation period is over. Four weeks after inoculation, seedlings are cut off 5 cm from the soil surface. The symptoms become clear on the new leaves, 7 to 10 days after cutting. Then the diseased seedlings are counted.

Of the varieties tested by this method, the following five are classified as resistant (Morinaka and Sakurai, 1970):

Kagura-mochi
Mangetsu-mochi
Saitama-mochi No. 10
Shinano-mochi No. 3
Tetep

Morinaka and Sakurai (1970) pointed out that a significant correlation was found between the percentage of diseased plants in the field and the percentage of diseased plants in the seedling inoculation. But the results obtained by the seedling test did not always agree with the field test. Resistant varieties classified by the field test sometimes showed high percentage of infection in the seedling test. Perhaps the seedling test method causes a more severe inoculation or perhaps there is a varietal difference in insect infestation in the field. In Taiwan, 70 varieties and selections have been tested by artificial inoculation. No-lin 49 and Taipei 131 did not show infected seedlings after inoculation (Chiu, Lin, and Huang, 1968). In Ceylon, H4 showed the lowest percentage of infected seedlings among eight varieties inoculated artificially (Abeygunawardena et al., 1970).

The resistance to the disease is a heritable character that has been known since Hashioka (1952) pointed it out. Recently, Morinaka, Toriyama, and Sakurai (1970) concluded that the resistance to yellow dwarf is controlled by a dominant or incompletely dominant major gene based on the reactions of F_1 to F_3 seedlings from crosses between Saitama-mochi No. 10 and two susceptible varieties, Manryo and Sanpuki.



Symptoms of yellow mottle disease.

YELLOW MOTTLE DISEASE

The disease was first observed near Kisumu along the shore of Lake Victoria in Kenya, in November 1966. The name rice yellow mottle was proposed for the disease by Bakker (1970) after he made an extensive study on it. The disease is not known to occur in other countries. All the following information on this disease is from Bakker's paper.

Symptoms

Yellow mottle is characterized by stunting and reduced tillering of the infected rice plant; crinkling, mottling, and yellowish streaking of the leaves; malformation and partial emergence of the panicles; and sterility. In severe cases, the infected plant may die.

The first symptoms appear about 7 days after inoculation. When seedlings of the rice variety Sindano are mechanically inoculated at the three- to six-leaf stage, the first newly formed leaves are mottled, streaked, and spirally twisted as well, as if they meet difficulty in emerging. The spirally twisted leaves can occasionally be seen in the field; leaves formed later are mostly normal in shape. When plants are inoculated at the eight- to 10-leaf stage, no malformation of the leaves occurs but the first symptoms consist of a

few yellow-green spots on the youngest leaves. These spots enlarge along the veins to give the characteristic streaking. Such leaves sometimes turn yellow and later become necrotic. Mottling of the leaf sheath also occurs. Many panicles do not emerge properly from the flagleaf sheath and are malformed with small, usually empty spikelets, resulting in greatly reduced production of viable seeds depending on the age of the plant at the time of infection. Sindano inoculated as late as 3 weeks before heading still shows a clear reduction in yield.

In the field the diseased plants are first noticeable 3 to 4 weeks after transplanting by their striking yellowish appearance. The youngest leaves have mottling or a mild yellow-green streaking. The plants are stunted, show reduced tillering, and the flowers are sterile.

Transmission

Yellow mottle can be transmitted to healthy rice plants by mechanical inoculation or by adult beetles *Sesselia pusilla* (Gerstaecker). No seed transmission was observed in experiments with a limited number of seeds from infected plants, however, nor was transmission obtained by growing rice plant in soil collected around diseased plants in the field.

The inoculum for mechanical inoculation can be prepared by cutting young diseased leaf blades into small pieces, grinding them in a mortar together with 0.01 M phosphate buffer, pH 7.0, at 1 ml/g leaf, and then squeezed the mixture through a muslin cloth.

Experiments on the transmission by adult beetles were made with either a single insect or a group of five insects. Ten beetles were transferred individually to rice seedlings each day after an acquisition feeding period of 4 days. Five of them transmitted the disease for one to five successive transfers. The symptoms appeared 9 to 16 days after inoculation feeding. Groups of five insects were placed on rice seedlings for 3 days. The surviving insects were transferred to another seedling for 3 days. In two tests, five out of seven and 12 out of 15 groups of beetles transmitted the disease during one of the two transfers. The symptoms appeared after 8 to 20 days. The presence of the virus in the beetles was checked serologically with the agar gel diffusion test.

In contrast to the feeding damage caused by caged beetles, damage to rice plants in the field due to the vector was never severe, although many of these insects were caught on ratoon rice. This suggests that the insects feed for short periods only.

The virus

The virus particles of yellow mottle are polyhedral, about 32 nm in diameter. The virus has a sedimentation coefficient (S_{20}) value of 116S.

The virus has been purified by grinding young diseased leaves in 0.1 M phosphate buffer, pH 5.0, at 20 ml/g leaf blade and squeezing the mixture through a muslin cloth. The sap is then mixed with chloroform at a ratio of 2:1 (v/v) and shaken gently for 5 minutes. The emulsion is centrifuged at 1,000 *g* for 10 minutes. Thereupon ammonium sulfate is added to the clear aqueous phase at the rate of 25 g/100 ml liquid while stirring with a magnetic stirrer. The precipitate is removed by centrifugation at 2,000 *g* for 15 minutes. Forty grams of ammonium sulfate is added per 100 ml of supernatant liquid. After standing for at least 20 minutes the solution is centrifuged at 4,750 *g* for 20 mintues and the precipitate resuspended in 3 to 6 ml of 0.1 M phosphate buffer. The suspension is dialyzed against 0.1 M phosphate buffer for 12 to 18 hours. The insoluble components are removed by centrifugation at 3,000 *g* for 20 minutes, leaving a strongly opalescent suspension which is fairly pure.

The virus can be further purified by either differential or density-gradient centrifugation. The preparation is centrifuged at 130,000 *g* for 90 minutes. The pellet is then resuspended in phosphate buffer. After standing for 30 minutes at 4 C, the insoluble components are removed by low speed centrifugation at 6,000 rpm for 3 minutes. The supernatant liquid is strongly opalescent and contained highly pure virus. For density-gradient centrifugation, the gradient column is prepared by placing in a tube 4, 7, 7, 7, and 3 ml of 0.01 M phosphate buffer, pH 6.7, which contains 0, 10, 20, 30, and 40 g sucrose per 100 ml, respectively. The preparation is layered on the top of the column and then centrifuged (SW 25.1 Spinco rotor) at 23,000 rpm for 100 minutes. A clear band appears at 9 to 13 mm below the meniscus. The band is then removed with a syringe and hypodermic needle and diluted five times with 0.01 M phosphate buffer, pH 6.7. After centrifuging at 133,000 *g* for 90 minutes, the pellet is resuspended in a small amount of 0.1 M phosphate buffer, pH 5.0.

The dilution end point of the virus depends on the source of sample. Sap from young rice leaves with clear symptoms, 2 to 3 weeks after inoculation, is still infectious at a dilution of 10^{-10}. The highest infectious dilution of sap obtained from plants inoculated 4 to 5 weeks earlier, was 10^{-6}. The thermal inactivation point of the virus is above 80 C. The virus in sap with 0.01 M phosphate

buffer, pH 7.0, remains infectious at room temperature (16 to 25 C) for 33 days but not for 51 days. When the sap is stored in a refrigerator (9 C), it is still infectious after 71 days. Chloroform, chloroform and butanol, and carbon tetrachloride and ether do not inactivate the virus.

An antiserum was prepared by injecting a rabbit with the purified virus. The highest dilution giving a reaction against crude sap and purified virus was 1/256 when determined by agar gel diffusion.

The virus has been recovered from sap obtained from the roots and also from the guttation fluid of diseased rice plants as well as from irrigation water from a heavily infected field. The explanation for the presence of the virus in irrigation water was that a fluid exudate often appeared on plant parts damaged by beetles, especially on stems, and this might be one of the ways in which the water becomes contaminated.

Host range

The rice varieties, Basmati, Basmati 217, Faya S1, Gamti, Kialangawa, Kibawa chekundu, Kibawa cheupe, Madevu, Mbuyu, Mkarafuu, Portugues, Shingo la Majani, Sindano, Uchuki, and Zira are susceptible to yellow mottle. In addition to rice, *Oryza barthii* and *O. punctata* are also susceptible. When the plants of these two species are infected, small yellow-green spots appear on the youngest leaves 14 days after inoculation. These spots extend along the veins. As the plants grow older, a slightly darker patch occurs in the center of the yellow streaks.

Other plant species such as *Avena sativa*, *Eleusine coracana*, *Hordeum vulgare*, *O. eichingeri*, *Pennisetum typhoideum*, *Sacharum officinarum*, *Secale cereale*, *Sorghum vulgare*, *Triticum aestivum*, *T. durum*, and *Zea mays* were not found to be infected after inoculation.

OTHER VIRUSES ON RICE

Mosaic of rice

Rice mosaic disease on variety Leuang Yai in the experimental field of the Bureau of Plant Industry in Manila was observed by Martinez et al. (1960) in 1960. Eight to ten percent of the rice plants were infected. The infected plants showed the characteristic symptoms of foliar mottling. The mottled areas were irregular in shape and varied in size from greenish dots to elongated yellowish green lesions. Some of these areas coalesced to form chlorotic streaks parallel to the vein. Mottling also occurred on the leaf sheath. In severe cases, the infected plants were stunted and produced very few tillers. The leaves were very much mottled. They gradually turned yellowish brown to brown and eventually withered. The tillers of the infected plants showed similar symptoms.

Using two mechanical inoculation methods, rubbing and pinprick, with the sap from diseased rice plants, Martinez et al. (1960) transmitted the disease to maize seedlings of the variety Cuban yellow flint. They suspected, however, that the mosaic disease of rice in the Philippines was closely related to, if not identical with, a mosaic disease that affects grasses. There is no information on the transmission of the disease from rice to rice or from maize back to rice, nor have any observations of the disease in the field been reported in the Philippines since 1960.

Barley stripe virus on rice

An isolate of barley stripe virus, mechanically inoculated to 2- to 3-week-old rice plants of 18 varieties and lines, caused systemic infection of seven varieties, PI 184675, PI 194676, PI 184676A, PI 201902, PI 201903, PI 201907, and PI 231126. The inoculum apparently was free of contamination with other viruses. The virus could be transmitted readily back to cereal plants from infected rice plants. None of the varieties was susceptible to the virus obtained from plants grown from infected seeds, however (Kahn and Dickerson, 1957).

Barley yellow dwarf virus on rice

A virulent strain of barley yellow dwarf virus obtained from Kent, England caused yellowing and stunting in other cereals. Host range studies showed that the virus can be transmitted to the rice variety GEB 24 by the aphid, *Rhopalosiphum padi* L. The infected rice

plants showed obvious symptoms similar to those in other cereals (Watson and Mulligan, 1960).

Brome mosaic virus on rice

Rice varieties PI 201902, PI 201903, and PI 231126 at 2 to 3 weeks old were susceptible when they were inoculated mechanically with two isolates of brome mosaic virus. Fifteen other varieties and lines tested were not infected. The virus could be readily transmitted back to cereal plants from rice plants showing systemic infection; none was recovered from the symptomless plants (Kahn and Dickerson, 1957).

Maize dwarf mosaic virus on rice

Rice varieties Arkrose, Bluebelle, Bluebonnet 50, CI 9205, CI 9534, and Nato were experimentally infected by mechanical inoculation with maize dwarf mosaic virus. There were no symptoms produced that could be related to infection of the rice plants. The infection was determined by assay on sorghum seedlings. The experimental results also indicated that the virus seemed to be localized in the inoculated leaves. Rice plants exposed to the virus under field conditions were not infected. Consequently, the virus may not be a potential problem to commercial rice production (Brambl and Dale, 1967).

Ryegrass mosaic virus on rice

The ryegrass mosaic virus transmitted by the eriophid mite, *Abacarus hystrix* (Nalepa), causes chlorotic streaks on the leaves of ryegrass. The virus was transmitted by manual inoculation of sap with celite to the rice variety CEB 24. One of the 16 inoculated rice plants became infected. The sap of infected rice plant was inoculated back to ryegrass for confirmation. One of the 10 inoculated ryegrass plants showed symptoms. The rice plant seemed to contain less virus than ryegrass and its sap did not precipitate specifically with antiserum prepared against the virus in ryegrass (Mulligan, 1960).

Sugarcane mosaic virus on rice

Four rice varieties, Bluebonnet 50, Colusa, Nato, and Rexoro, were first found to be susceptible when inoculated with strain H of sugarcane mosaic virus by the air-blast method (Anzalone, 1963). The mosaic symptoms on rice were somewhat milder than those on

sugarcane but the rice plants still showed a typical mosaic pattern of pale or light green elongated patches in the dark green tissue of the leaves. The virus could be transmitted readily back to sugarcane. Abbott and Tippett (1964) demonstrated that rice varieties Bluebonnet 50, Caloro, Colusa, Gulfrose, Horai, Nato, and Rexoro were susceptible to strain H of the sugarcane mosaic virus. Caloro Gulfrose, and Horai were susceptible to strain D and Horai, also to strain A. Recently Anzalone and Lamey (1968) reported that rice varieties Berlin, British Guiana No. 79, Jojutla No. 721 (PI 245717), and Pandhori No. 4 showed promise for differentiating strains A, B, D, and H of the sugarcane mosaic virus.

ADDENDUM

After the manuscript of this publication was completed, M.S.K. Ghauri published a paper "Revision of the genus *Nephotettix* Matsumura (Homoptera: Cicadelloidea: Euscelidae) based on the type material" (Bull. Entomol. Res. 60:481-512, 1971). Ghauri listed eight valid species and one subspecies in the genus *Nephotettix*. Their trivial names are *N. afer* Ghauri, *N. cincticeps* (Uhler), *N. malayanus* Ishihara & Kawase, *N. modulatus* Melichar, *N. nigropictus* (Stål), *N. nigropictus yapicola* Linnavuori, *N. parvus* Ishihara & Kawase, *N. sympatricus* Ghauri, and *N. virescens* (Distant).

Ghauri pointed out that the type-specimen of *Pediopsis apicalis* de Motschulsky and *P. nigromaculatus* de Motschulsky have been destroyed. But the holotype male and allotype female of *N. nigropictus* provided a well-defined and authentic concept which is the same as *N. apicalis*. The former name should, therefore, replace the latter.

He also pointed out that *Selenocephalus virescens* Distant in the collection of the British Museum belongs in *Nephotettix*, and a comparison of the specimen with the holotype of *Cicada bipunctatus* Fabricius left no doubt as to their being conspecific. Since *C. bipunctatus* is preoccupied, the next name in seniority is *N. virescens* (Distant) which therefore replaces *N. impicticeps* Ishihara.

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INDEX

CORRECTIONS

p. 12. line 5. Change / their feeding tracks / to:
its feeding tracks

p. 12. line 12. Change / devise / to:
device

p. 13. line 6. Change / early / to:
earliest

p. 28. line 5 from bottom. Change / medically / to:
medially

p. 32. line 22. Change / time / to:
times

p. 35. line 2. Change / serves a guide / to:
serves as a guide

p. 37. line 36. Should read:
Another example occurred in Mindanao a few years ago when

p. 38. line 15. Change / a run / to:
a run of

p. 44. line 34. Change / it does not / to:
the test does not

p. 46. line 32. Change / seedlings we inoculate / to:
we inoculate seedlings

p. 48. line 31. Change / varieties to the disease / to:
varieties in reaction to the disease

p. 74. line 12 under Host range. Change / that of hoja blanca / to:
that hoja blanca

p. 80. line 5. Change / or well-defined / to:
or more well-defined

p. 111. line 4. Change / other days / to:
other day

p. 115. line 10 under Transmission. Change / squeezed / to:
squeezing

p. 120. After last paragraph. Add:

Another paper "Three new beetle vectors of rice yellow mottle virus in Kenya" by W. Bakker (Neth. J. Plant Pathol. 77:201-206, 1971) reported that *Chaetocnema pulla* Chapuis, *Trichispa sericea* (Guérin), and *Dicladispa (Chrysispa) viridicyanea* (Kraatz) are vectors of yellow mottle virus. The virus-vector interaction of these three species seems to be similar to that of *Sesselia pusilla*.