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RICE VIRUS DISEASES TRANSMITTED BY BROWN PLANTHOPPER IN TAIWAN WITH SPECIAL REFERENCE TO RICE WILTED STUNT **【Research report】**

在台灣褐飛蝨傳播之水稻萎凋病及其他毒素病之研究 **【研究報告】**

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Abstract

Five different virus or mycoplasma-like diseases of rice have been reported in Taiwan. They are yellow dwarf (RYD) (28), transitory yellowing (RTY) (20), stripe (25), ragged stunt (RRS) (14), and grassy stunt (RGS) (26) and RGS related diseases (12). The causal agents of the first two diseases are transmitted by rice green leafhoppers of *Nephotettix* spp. (18,19,20,27), and that of stripe is transmitted by small planthopper, *Laodelphax striatellus* (Fall.) (24,25). RRS and RGS are transmitted by the brown planthopper (BPH), *Nilaparvata lugens* Stal (11,14,26). In 1977-1978, three BPH-borne diseases of rice with virus-like symptoms were collected in central Taiwan (12,15). Based on vector relationship, characteristics of symptoms and cellular inclusion (16), these diseases were considered as grassy stunt related diseases (12,16,30). RRS was first found in Chiayi in 1979 (14), later, it was also observed in Taichung area with sporadic occurrence. This paper will review some results both from laboratory and field studies conducted in Taiwan on BPH-borne diseases. An extensive study has been made on grassy stunt related diseases, particularly the one which has named as rice wilted stunt. Some of the results which have not been published are also discussed in this paper.

摘要

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Rice Virus Diseases Transmitted by Brown Planthopper in Taiwan with Special Reference to Rice Wilted Stunt

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ABSTRACT

Five different virus or mycoplasma-like diseases of rice have been reported in Taiwan. They are yellow dwarf (RYD)⁽²⁸⁾, transitory yellowing (RTY)⁽²⁰⁾, stripe⁽²⁵⁾, ragged stunt (RRS)⁽¹⁴⁾, and grassy stunt (RGS)⁽²⁶⁾ and RGS related diseases⁽¹²⁾. The causal agents of the first two diseases are transmitted by rice green leafhoppers of *Nephotettix* spp.^(18,19,20,27), and that of stripe is transmitted by small planthopper, *Laodelphax striatellus* (Fall.)^(24,25). RRS and RGS are transmitted by the brown planthopper (BPH), *Nilaparvata lugens* Stål^(11,14,26). In 1977-1978, three BPH-borne diseases of rice with virus-like symptoms were collected in central Taiwan^(12,15). Based on vector relationship, characteristics of symptoms and cellular inclusion⁽¹⁶⁾, these diseases were considered as grassy stunt related diseases^(12,16,30). RRS was first found in Chiayi in 1979⁽¹⁴⁾, later, it was also observed in Taichung area with sporadic occurrence.

This paper will review some results both from laboratory and field studies conducted in Taiwan on BPH-borne diseases. An extensive study has been made on grassy stunt related diseases, particularly the one which has named as rice wilted stunt. Some of the results which have not been published are also discussed in this paper.

Rice Grassy Stunt

Grassy stunt was originally reported from Philippines⁽³¹⁾ and it was first observed in a greenhouse at National Chung Hsing University in Taiwan⁽²⁶⁾. However, the occurrence of the disease in the field was not certain at that time. The infected plants were characterized by severe stunting, excessive tillering and erect growth. The leaves were pale green or pale yellow with numerous rusty spots of various sizes or in blotches. Grassy stunt virus was transmitted by BPH in a persistent manner. After allowing a 24 hr acquisition feeding on diseased plant, about 50% of BPH became transmitters. The incubation period of the virus in the insect ranged from 6-20 days with an average of 9.5 days. The disease agent was neither transovarially transmitted nor through soil, seed or by mechanical transmission.

Recently, the cause agent of RGS was found

as filamentous virus by Hibino⁽³³⁾.

Grassy Stunt Related Diseases

In 1977-1978, three different diseases with virus-like symptoms were collected in the central Taiwan. Chen *et al.* demonstrated that these diseases were transmitted by BPH^(5,7,15). Of these, the most severe type, characterized by extreme plant stunting, leaf wilting and premature plant death was named as GSB and GSY⁽¹²⁾. These diseases were later considered to be related to grassy stunt, on the basis of vector relationship, type of cellular inclusions and stimulation of tillering in some of the test varieties. Recently, an unknown disease transmitted by BPH was reported in Philippines⁽¹⁾. The causal agent of the disease was shown to have relationship with RGS⁽²¹⁾. According to their descriptions of the symptoms, the disease is probably identical with rice wilted stunt.

Rice Wilted Stunt

Symptom

The major characteristic symptoms of rice affected by rice wilted stunt (RWS) were severe plant stunting, reduced or increased tillering and discoloration of leaves ranging from orange to pale yellowing. The symptoms, however, varied considerably with the rice variety, the environmental condition and the growth and age of the plant.

On experimentally infected plants of Taiwan 5 (a Japonica rice), the symptoms began with a rusty spotting and yellowing of the lower basal leaves and paling of the central, newly unfolded leaves discernible about 10 days after inoculation. The disease thus resembled transitory yellowing at this stage. The rusty spotting and yellowing of leaves later intensified, and the plants wilted. Infected plants produced small tillers, and the number of tillers was reduced in winter but increased slightly in summer. In winter, most disease plants died at 30-50 days after inoculation. Those that did not die at this time produced small, pale leaves and remained extremely stunt. Leaf twisting and trapping of unfolded leaves usually appeared about 20-25 days after inoculation. Symptoms were generally milder and the disease was not lethal under summer greenhouse conditions, although infected plants died in the field during the second crop season.⁽¹⁶⁾

On TN 1 (an Indica rice), the RWS-infected plant was markedly stunted. Leaves appeared pale green, shortened, narrower than normal, and usually curled inward and was brittle. The number of tillers was not affected in winter but increased by several times in summer. The infected plants produced none or only a few, poorly developed panicles.

Insect Transmission

Rice wilted stunt virus (RWSV) was experimentally proved to be transmitted by BPH in a persistent manner. Chen *et al.*^(15,15) reported that about 41% of the test insects were able to transmit the virus. The percentage of active transmitter was affected by temperature and

other factors⁽¹³⁾. There were 12, 15, 45, 44 and 39% active transmitter when the insects were allowed to feed on the disease plant at 15, 20, 25, 30 and 35C, respectively. Both nymph and adult could acquire the causal agent and transmit the disease. However, the nymphs were found more efficient to acquire the virus than adults. Besides, the former had a shorter incubation period. When nymphs of BPH were allowed to feed on different parts of the same diseased plant, more insects became viruliferous when fed on leaves (23%) than on stem (16%) or root (14%). When nymphs of BPH were allowed to feed on the diseased plant at 10, 30, 60 and 120 days after symptom appearance, 4, 27, 24 and 15% of the insects became viruliferous, respectively. Thus the diseased plant at 30 and 60 days after symptom appearance may have possessed a higher concentration of virus compared with younger or old diseased plants.

The minimum acquisition and inoculation feeding periods were 2 and 1 hour, respectively. The incubation period in insect vector was from 3 to 14 days with an average of 6.7 days at 28-30C. The incubation period in insect vector after acquisition feeding was determined as 28.5, 19.5, 11.9, 8.6, 7.0 and 5.8 days at 15, 20, 24, 27, 30 and 35C, respectively. The threshold temperature for BPH to acquire the virus and to complete the incubation period was 12C.

RWSV was neither transovarially transmitted nor transmitted by other rice hoppers insects, such as *Nephotettix* leafhoppers, *Inazuma dorsalis*, *Laodelphax striatellus* and *Sogatella furcifera*. In addition, there was no evidence so far for the transmission of RWSV through seeds, soil or by mechanical methods.

The Causal Agent

Electron microscopic observation of thin section of RWS infected plant tissues revealed the presence of bundle-shaped inclusion, comprised of filaments of different lengths, in both the phloem and mesophyll cells⁽¹⁶⁾. Similar structures were previously found by Pellegrini and Bassi⁽³⁰⁾ in rice plants infected with grassy stunt. No detailed studies on the morphology of the virion of RWSV has been made in Taiwan.

Physio-Chemical Properties of the Virus

Chen (1984)⁽⁹⁾ reported the virus retained its infectivity in crude leaf extracts in 0.1M phosphate buffer at PH values between 5 and 9. The *in vitro* longevity of the virus in rice leaf sap was 5 days at 4C and only one day under room temperature (26-33C). The dilution end point was between 10^{-4} - 10^{-5} in crude plant sap and was 10^{-6} - 10^{-7} in insect extracts. The thermal inactivation point in rice leaf extracts was 50C for 10 minutes. The infectivity of the virus was not reduced by three cycles of freezing-thawing of diseased leaf extracts.

Effect on Plant Growth and Yield

Rice wilted stunt disease has a significant influence on rice plant growth and yield. The degree of plant stunting and number of tillers affected by the disease varied with plant age and season at time of infection and with rice varieties involved⁽⁶⁾. For example, when Tainan 5 was inoculated at the seedling stage, the height of the infected was only 25 to 40% of the normal one. The effect on plant height reduction was decreased as plant age for inoculation increased. Reduction of the number of tillers is another characteristic of this disease. Tiller numbers of infected Tainan 5 plants were reduced under winter greenhouse condition, and were slightly increased during summer season, but for some test varieties such as Taichung 65 and TN 1, they were slightly increased in winter and were markedly increased during the summer season.

The major yield components such as panicle number, length of panicle, percent of filled grains and weight of one thousand grains of the diseased plant decreased in proportion to the age of the rice plant at time of infection. During the field trials made in the second crop season, infection with RWS resulted in 94, 78, 58 and 39% yield reduction for Tainan 5 when inoculated at 30, 40, 50 and 60 days after germination. Infection with RWS resulted in 96, 59 and 42% yield loss for Taichung Sen 3 when inoculated at 30, 40 and 50 days after germination. With these varieties, there was no yield at all when inoculation was made within 30 days after germination.

Premature death is another characteristic of RWS on rice plant. In the 2nd crop season, the percentage of premature death was 53 to 100 and 54 to 100, respectively, for Tainan 5 and Taichung Sen 3 when inoculated before 30 days after germination. No diseased plants died prematurely when these varieties were inoculated at 40 to 50 days after germination.

Epidemic of RWS

1. Host plant

RWSV experimentally infected *Oryza sativa* and other three species of wild rice, *O. nivara*, *O. barthii* and *O. stapfii*. None of the other 13 species of *Gramineous* plants tested were found infected⁽⁸⁾.

2. Latent period in plant

Chen (1981)⁽⁶⁾ reported the latent period of RWSV in rice plant was 10 to 14 days during summer season, but it required from 15 to 20 days during cooler season (from February to April). The latent period was prolonged when older plants were inoculated. For example, the latent period in Taiwan in the field of 2nd crop, 1979 was 12 to 15 days when inoculated before 30 days after germination, but it required 23 to 30 days when inoculated at 40 to 60 days after germination.

3. Fluctuation of the virus source

During the period of 1979-1983, about 17000 BPH were collected in each 15-day intervals from paddy fields in Tung-Shih, Taichung and tested individually for virus carriers. No viruliferous insects were found in the collections made in December through May. The viruliferous insects were detected first in June or July, at 0.3-1.6% and the active transmitters reached a peak during September to November, with 3.3-7.5% of the field population carrying RWSV⁽⁸⁾ (Fig. 1).

When rice plants were transplanted at 10 days intervals in the field and allowed to be infected naturally, the disease incidence paralleled with the seasonal fluctuations of viruliferous insects. No plants were found to be infected with RWSV in December through May. Infected plants started to appear in June or July and new infection to be detectable up to October or November at an incidence level of 0.1-2.3%⁽⁸⁾

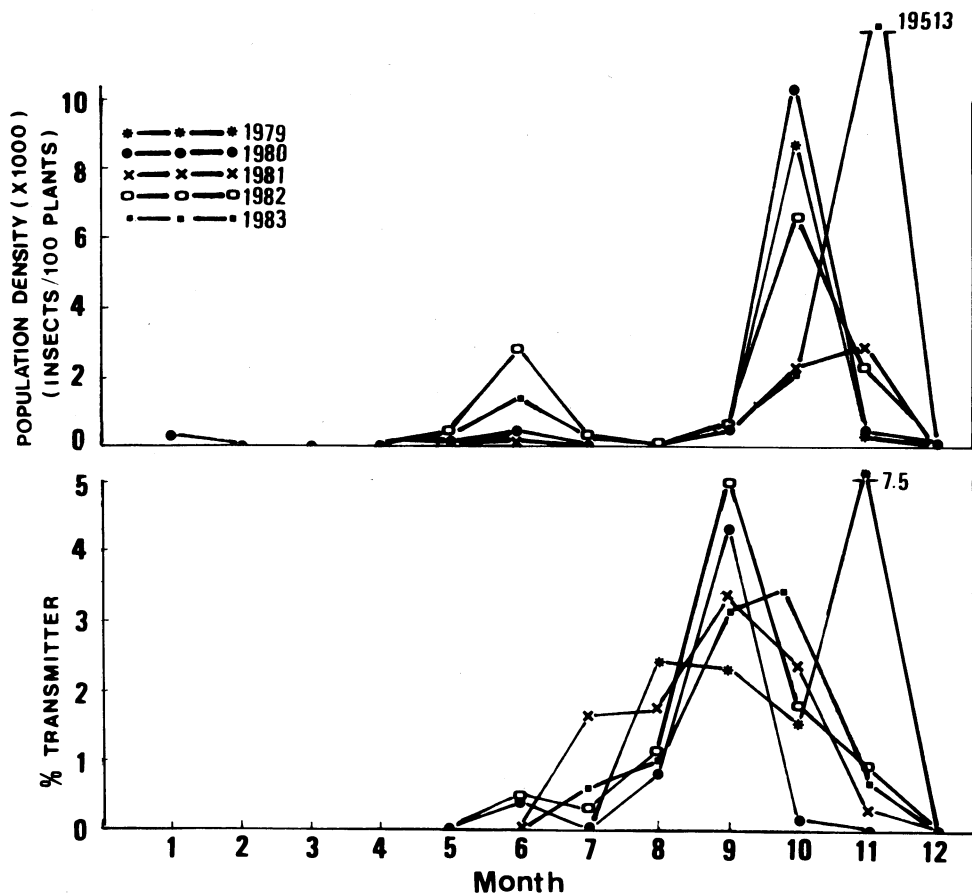


Fig. 1. Seasonal changes of the population density of brown planthopper (adults) and the percentages of insects carrying RWSV as determined in 1979-1983 (Chen, 1984).

4. Time of RWSV infection in the field

Disease survey conducted in the field of Tung-Shih, Taichung beginning 10 days after transplanting at 10-days intervals showed that the incidence of RWS ranged from 0.8 to 4.5% in the 2nd crop season during 1979-1983. The diseased plants commenced to appear 20 to 90 days after transplanting. Based on the latent period of RWSV in the rice plant⁽⁶⁾, it is suggested that infection of RWSV in the field might

disperse evenly from the seedling stage to the booting of the rice plants.

Source of Resistance

During 1982-1983, a total of 264 varieties/lines of rice plant, *Oryza sativa* and three wild rice *O. nivara*, *O. barthii*, and *O. stapffi* were tested and inoculated at the seedlings stage for their resistance to RWSV under greenhouse

conditions. Varieties such as IR2261-464-2, IR1541-76-3, IR1541-102-7, PC-5 and Chai-Nung-Shi-Bi 662070 showed highly resistant to RWSV in 3-5 separate tests (Chen, unpublished data).

GSB

The characteristic symptom of GSB on Tainan 5 was excessive tillering, which was noticeable from early stages of disease development. Number of tillers were nearly doubled in winter and as much as seven times that of healthy plants in summer. Young leaves of diseased plants appeared green and usually had vague, chlorotic stripes on both sides of the midrib. Later, the leaves turned pale green with conspicuous mottling. Diseased plants that were mildly stunted usually lived to maturity but produced only a few filled panicles. The ratooning ability was not impaired. Effects of GSB on Taichung 65 and TN 1 were similar to those on Tainan 5, except that diseased plants were less stunted. GSB was proved to be transmitted by BPH. The incubation period of the virus in the insects ranged from 4 to 23 days, with an average of 8.7 days. Other characteristics of transmission are similar to those of RWSV⁽¹²⁾.

GSY

In both Tainan 5 and Taichung 65 rice plants infected with GSYV, tillering was reduced slightly in winter but increased slightly in summer. Plants were less stunted than those infected with RWSV. New leaves of GSYV infected plants appeared pale green and mature leaves had a normal green color. Rusty yellowing often developed on older basal leaves. Leaf width and stem diameter were not affected by the disease. Although infected plants lived to maturity, they failed to head. The first visible symptom of GSY on TN 1 was a paling of young leaves. Diseased plants were markedly stunted, with a nearly normal tiller number in winter but an increase by several times in summer. Diseased plants produced narrow, stiff leaves in an erect growth habit. There also were numerous small, dark brown spots on the leaves. Diseased plants lived to maturity but usually produced empty panicles.

GSY was also found to be transmitted by BPH with an incubation period of 7.9 days (ranged 4-17 days). Other characteristics of transmission are also similar to those of RWSV⁽¹²⁾

Rice Ragged Stunt

Rice ragged stunt (RRS) was originally discovered in Indonesia^(2,21) and in Philippines⁽²⁹⁾. In Taiwan, it was found in the paddy field of Chayi in 1978⁽¹⁴⁾. Symptoms included stunting of plants with leaves slightly darker in color than normal and brittle in feeding; the leaves became twisted and ragged. A few vein-swells were found on the lower surface of leaf blades and outer surface of leaf sheaths, if any, had mostly unfilled grains. The latent period for symptom appearance was 8-10 days in summer but extended to more than one month in winter. The BPH transmitted the virus with an average incubation period of 9.6 days (ranged 5-14 days). About 22% (ranged 15.2-30.4%) of the insect vectors were active transmitters. The minimal period for acquisition feeding was 2 hr, whereas minimal inoculation period was 1 hr. Efficiency of virus acquisition increased with increased temperature. The percentages of virus acquisition were 1.8, 16.1, 21.8, 19.6 and 26.8 when the insects were allowed assessing 24 hr on diseased plants at 15, 20, 25, 30 and 35C, respectively. The incubation period in the insect vector was also affected by the temperature, the average of incubation period were 16.9, 10.8, 7.4, 7.5 and 6.0 days at 20, 25, 28, 30 and 35C, respectively.

The Virus

The causal agent of rice ragged stunt has been suggested as a reo-like virus^(23,32). Some studies on the morphology of the virus have been made in Taiwan⁽¹⁰⁾. Electron microscopic observation of ultrathin section of the infected plants revealed that the particles of RRSV were embedded in viroplasm inclusion in the cytoplasm of phloem cells. Particles were about 66 nm in diameter and electron dense cores of about 45 nm were surrounded with less electron dense shells.

Thin sections of insect transmitters also

showed that virus particles were embedded in viroplasm inclusion in cytoplasm or arranged in tubular structure scattered in the cells of antenna, compound eye, muscle, skin, fat bodies,

gastric caeca, desophagus, mid-gut, hind-gut, Malpighian tubules and salivary gland of the viruliferous insects. (Fig. 2)

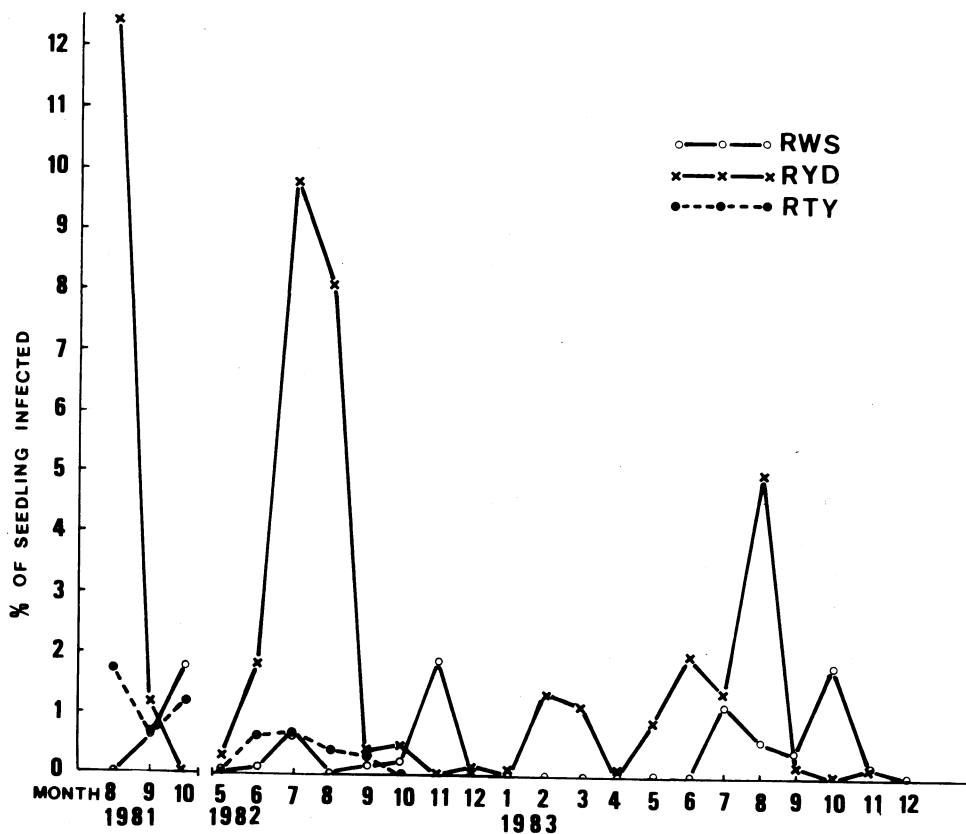


Fig. 2. Infection of rice plants with rice wilted stunt (RWS), rice yellow dwarf (RYD) and rice transitory yellowing (RTY) following an exposure at the seedling stage to the field populations of the insect vectors in different months at Tung-Shih, Taichung.

Discussion

The virus or virus-like diseases transmitted by the green leafhoppers (GLH) or brown planthoppers (BPH) occur primarily on the second crop (July to November) of rice plants in

Taiwan⁽¹⁷⁾. In term of acreage and severity of damage, diseases such as yellow dwarf and transitory yellowing transmitted by GLH are more serious than those such as wilted stunt and ragged stunt which are transmitted by BPH (Fig. 3). In central Taiwan, this may be explained

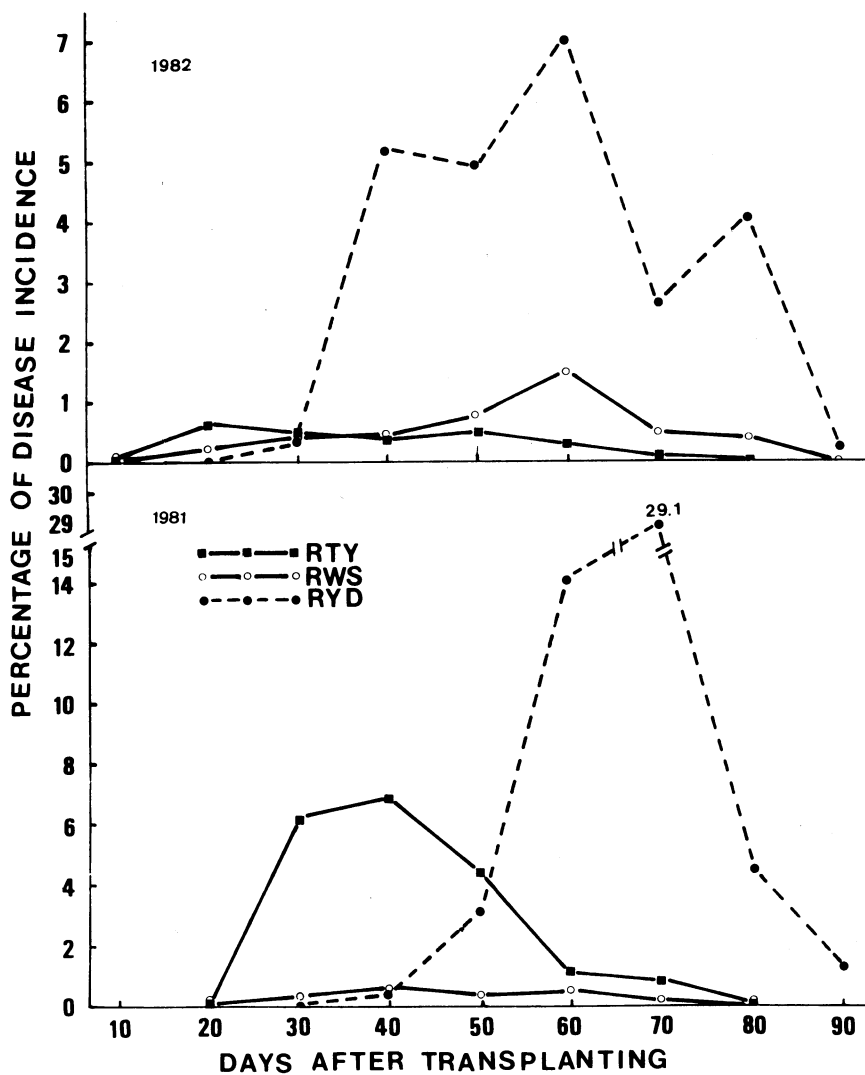


Fig. 3. Comparing the incidence of rice transitory yellowing (RTY), rice wilted stunt (RWS) and rice yellow dwarf (RYD) at different growth stage of the 2nd rice crop in 1981 and 1982.

in part by the ecological differences observed for GLH and BPH and by the conditions of inoculum source fluctuation^(2,3,4,81).

Population of BPH could be found in the field during the first rice crop (February to July) and at certain locations for some years, BPH has caused serious damage on rice plants during later period of the first crop, yet, no virus diseases

transmitted by BPH have actually been found⁽⁸⁾. This may have due to a lack of virus sources (Fig. 1 & 2), since neither viruliferous insects nor diseased plants could be detected in the fields from December to May during 1979 to 1983. On the other hand, 64% or high rate of GLH on the seedling stage have been found as carriers of the causal agent of yellow dwarf (RYD) in the 1st

crop^(2,3). This directly or indirectly causes high incidence of RYD during the later stage of the first crop or on the ratoon rice. The diseased plants and the insect vectors carrying the disease agent become the important sources of inoculum for the following crop.

Although population of GLH (4th generation) in the field usually starts to decrease following the harvest of the 1st crop, with plenty of inoculum sources and high percentage of insects carrying disease agent, infection of rice plants by RYD is readily found at seedling bed and in the early paddy field on the 2nd crop. On the contrary, both the population of BPH and viruliferous BPH at early stage of the 2nd crop are low. Furthermore, there is generally a lack of virus source at this time. All these factors contribute to a reduced early infection of rice by

RWSV. The active transmitters and disease incidence (Fig. 1 & 2) reach a peak stage during September to November, in addition, the population of BPH also increase, however the rice plants at this stage have already possessed resistance to the infection. The infected plants may not show any symptoms, and the yield is not significantly affected⁽⁶⁾. A diagram for comparing the GLH and BPH transmitted diseases is given in Fig. 4.

Based upon the above mentioned ecological observations of insect vectors and virus source fluctuation, it is believed that the diseases transmitted by BPH will not impose serious threat to rice production with current culturing practice in Taiwan. In view of the findings stated above, however, green rice leafhopper and the diseases it transmits should not be neglected.

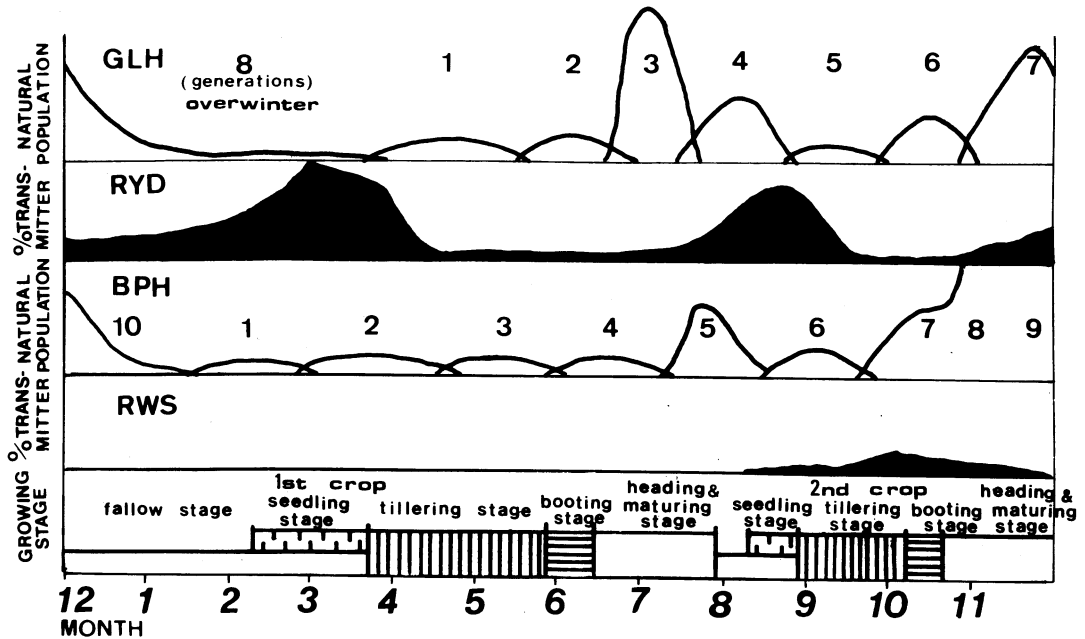


Fig. 4. A diagram illustrates the seasonal changes of the disease of rice yellow dwarf (RYD), rice wilted stunt (RWS) and their insect vectors in the central Taiwan.

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