Three Symptomatologic Types of Rice Virus Diseases Related to Grassy Stunt in Taiwan

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ABSTRACT

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Three rice diseases with viruslike symptoms were collected from central Taiwan. The diseases were designated as wilted stunt, grassy stunt B, and grassy stunt Y. Their causal agents were all transmitted by the rice brown planthopper *Nilaparvata lugens* in a persistent manner, with incubation periods in the vector ranging from 3 to 23 days. Symptomatologically, wilted stunt was distinct in that it caused extreme plant stunting and was often lethal to rice plants, especially in the winter months. Both grassy stunt B and grassy stunt Y also caused stunting, but they were not lethal to test plants. All three disease types stimulated tillering in some rice cultivars in summer; only grassy stunt B had this effect in winter. On the basis of vector-relationships and enhancement of host tillering, these three diseases were tentatively identified as virus diseases related to rice grassy stunt.

Additional key words: planthopper transmission, *Oryza sativa*, virus isolates

The rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV) are transmitted by the rice brown planthopper *Nilaparvata lugens* (Stål). Recent work has shown that RGSV has isometric particles 20 nm in diameter (17) and that RRSV has reoviruslike particles 63–65 nm in diameter (9,18). Both are transmitted in a persistent manner with an incubation period in the vector ranging from 6 to 20 days for RGSV (11,15) and 5 to 18 days for RRSV (8,14). The two diseases are easily separable by symptoms (1,11,14,15).

Grassy stunt disease of rice was first recorded in Taiwan in 1970 (11). In 1977–1978, several diseases on rice with viruslike symptoms were collected from various locations in the central part of Taiwan during the second crop season. Among these, a disease characterized by extreme plant stunting and leaf wilting and by transmissibility of the causal agent by the rice brown planthopper was tentatively named rice wilted stunt (2). Affected rice plants showed little tendency toward excessive tillering, as is normally seen in plants diseased with grassy stunt (11,13,16). The causal agents of the other disease types were found to be transmissible by the brown planthopper. These findings prompted us to make further comparisons of host varietal responses and vector relationships for these diseases. Results of these comparisons are reported here.

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MATERIALS AND METHODS

The wilted stunt disease (GSW), a 1977 collection from a rice field in Tungshih in the Taichung area (2), was maintained in rice plants by periodic transfers to new plants using *N. lugens* as vector. Two other symptom types, designated grassy stunt B (GSB) and grassy stunt Y (GSY), were collected in 1978 from Chiayi and Shikon, respectively, also in the central part of Taiwan, and maintained in rice plants as above.

Rice cultivar *Oryza sativa* 'Tainan 5,' a Japonica type, was used in all transmission experiments. Cultivars Taichung 65 (a Japonica), Taichung Native 1 (TN 1), Taichung Sen 3, and Shan-san-sa-san (all of Indica type) were used for symptom comparison among the diseases. According to Ling et al (12), Shan-san-sa-san responds to grassy stunt by producing yellowish white leaf stripes and is an indicator plant for the disease.

Rice brown planthoppers were maintained in cages (55 × 55 × 90 cm) on Tainan 5 plants under continuous fluorescent lighting at 26–28 C. Healthy cultures were established by confining adult females on *Echinochloa crus-galli*, on which the planthopper laid eggs but could not complete the life cycle. Eggs hatched in 7–10 days at 28–30 C, and the desired instars were transferred to healthy rice plants. Insects were sampled at random and tested on healthy rice seedlings monthly to assure that the cultures were virus-free. Other test insects were similarly maintained on Tainan 5 plants.

In transmission experiments, acquisition feeding was usually performed by enclosing second to third instars on diseased plants for 24 hr at 28–30 C in glass cylinders (10 × 30 cm) covered with nylon screen. Test insects were transferred to two-leaf, healthy seedlings in test tubes daily or at 2-day intervals following acquisition feeding until the insects died. In symptom-comparison experiments, plants of all test cultivars were inoculated simultaneously. Inoculated plants were kept in the greenhouse for the development of symptoms at temperatures that ranged from 20 to 40 C, depending on the season. During the winter, supplemental lighting from incandescent lamps was provided.

RESULTS

Symptoms. GSW appeared to be more severe than the other two disease types. Characteristic symptoms on Tainan 5 included 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 (7) at this stage. The rusty spotting and yellowing of leaves later intensified, and the plants wilted. Infected plants produced small tillers, the number of which was reduced in winter but increased slightly in summer.

Under winter conditions, most diseased plants died 30–50 days after inoculation. Those that did not die at this time produced small, pale leaves and remained extremely stunted. Leaf twisting and trapping of unfolded leaves were characteristic of GSW on this cultivar, usually appearing about 20–25 days after inoculation (Fig. 1A). Symptoms were generally milder and the disease not lethal under summer greenhouse conditions, although infected Tainan 5 plants died in the field during the second (summer) crop season.

Taichung 65 was similarly affected by GSW, but stunting was less severe and tillering was increased slightly in winter and by two to three times in summer.

TN 1 was markedly stunted by GSW (Fig. 1C and D). Leaves appeared pale green, shortened, narrower than normal, and usually curled inward and were brittle. Tillering was not affected in winter but increased by several times in summer. Some of the diseased plants died prematurely, particularly in winter; others survived but produced no panicles or only a few, poorly developed ones.

On the cultivar Shan-san-sa-san,

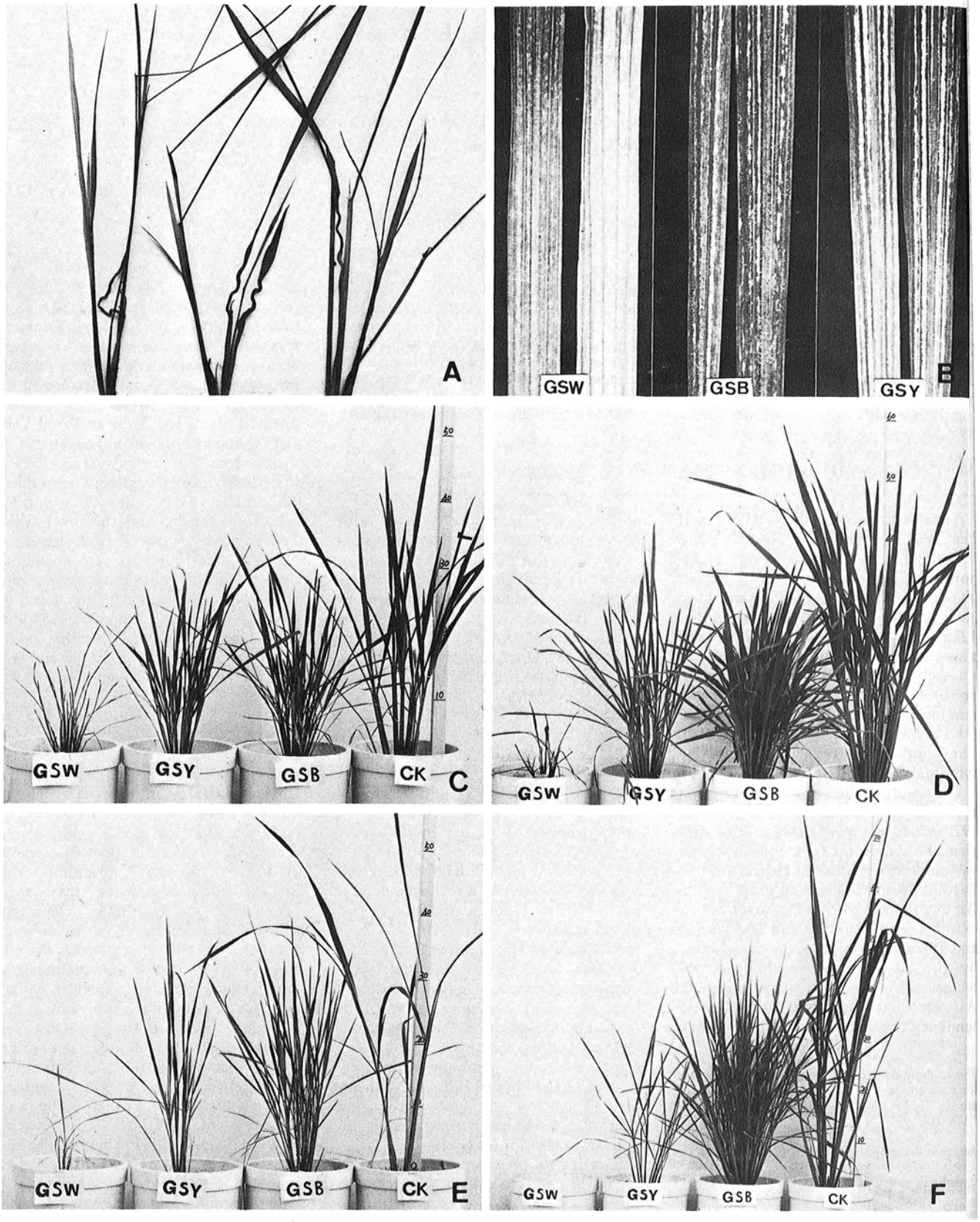

Fig. 1. Symptoms of three diseases related to grassy stunt on rice cultivars under winter greenhouse conditions. GSW = wilted stunt, GSB = grassy stunt B, and GSY = grassy stunt Y. **(A)** Leaf twisting and trapping caused by GSW on cultivar Tainan 5. **(B)** Whitish yellow leaf streaks and stripes on cultivar Shan-san-sa-san. Growth of cultivar TN 1 at **(C)** 60 and **(D)** 90 days after inoculation, and of cultivar Shan-san-sa-san at **(E)** 60 and **(F)** 90 days after inoculation. Note the differences in amount of stunting and tillering.

young leaves of diseased plants remained pale green to pale yellow and exhibited conspicuous mottling about 3 wk after inoculation. Sometimes vague, chlorotic stripes formed on leaves along the veins (Fig. 1B). However, these became masked about 30 days after inoculation. Diseased plants were extremely stunted and had fewer tillers than healthy plants; eventually leaves dried from basal positions, and the plants died (Fig. 1E and F). Premature death and reduced tillering were also observed on another Indica type cultivar, Taichung Sen 3.

The characteristic symptom of GSB on Tainan 5 was excessive tillering, which was noticeable from early stages of disease development. Numbers 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 (Fig. 1C and D).

Shan-san-sa-san plants affected with GSB showed excessive tillering and stunting (Fig. 1E and F), and young leaves developed conspicuous short streaks in winter that later fused to form stripes along the veins (Fig. 1B). As diseased plants aged, leaves turned yellow and the stripes became vague. No such stripes could be seen in summer. Leaves of the diseased plants appeared pale green to yellow and were conspicuously narrower than normal. Diseased plants produced small panicles with normally filled grains. Of the three disease types, GSB appeared to cause the greatest increase in tillering.

In both Tainan 5 and Taichung 65 plants affected with GSY, tillering was reduced slightly in winter but increased slightly in summer. Plants were less stunted than when diseased by GSW. New leaves appeared pale green and mature leaves had a normal green color. Rusty yellowing often developed on the 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 (Fig. 1C and D) 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 produced empty panicles.

On the variety Shan-san-sa-san, GSY caused conspicuous yellowish white stripes along the leaf veins (Fig. 1B). The tiller number was slightly reduced in winter and greatly increased in summer.

Transmission. Three rice leafhoppers (*Nephotettix cincticeps, N. nigropictus,* and *Recilia dorsalis*) and three planthoppers (*Nilaparvata lugens, Laodelphax striatellus,* and *Sogatella longifurcifera*) were tested for ability to transmit the causal agents of the three disease types. For each disease type, 40–50 individuals of each test insect were used. After a presumed incubation period of 10 days, individual insects were serially transferred to test plants. Only those seedlings fed on by *N. lugens* developed disease symptoms, indicating a vector role for the brown planthopper. Insect infectivity persisted until death.

Results of several transmission tests showed incubation periods of 3–14, 4–23, and 4–17 days, with averages of 6.3, 8.7, and 7.9 days for the GSW, GSB, and GSY agents, respectively (Fig. 2).

DISCUSSION

Five different virus or mycoplasmalike diseases of rice have been reported from Taiwan. They are yellow dwarf (5), transitory yellowing (7), stripe (10), grassy stunt (11), and ragged stunt (1). The causal agents of the first two are transmitted by rice green leafhoppers of *Nephotettix* spp. (5–7), and that of stripe is transmitted by the small planthopper *Laodelphax striatellus* (10). RGSV and RRSV are transmitted by the planthopper *Nilaparvata lugens*.

In this study, *N. lugens* transmitted the causal agents of GSW, GSB, and GSY. The characteristic symptoms of ragged stunt, which include ragged leaves and formation of vein galls on leaf sheaths (1,14), are quite distinct. The question thus remains whether the GSW, GSB, and GSY agents are strains of RGSV. Hsieh and Chiu (11) reported the occurrence of grassy stunt in Taiwan, but the virus culture has not been maintained. Quarantine regulations preclude the possibility of comparing GSW, GSB, and GSY with grassy stunt originally reported from the Philippines.

The severity of symptoms varied with host variety and season. Although increased tillering was consistently associated with GSB, the stimulating effect was greater in summer. GSY did not affect tillering in winter, but greatly increased it under summer greenhouse conditions. GSW-diseased plants generally had fewer tillers in winter, but host reactions in summer varied with test cultivars. Hsieh and Chiu (11) noted that enhanced tillering of plants diseased with grassy stunt may not be expressed at low temperatures or with an inadequate supply of fertilizer.

Symptomatologically, GSW appears distinctive in that it caused the most severe plant stunting, twisted and trapped leaves, and premature plant death. Such severe symptoms have not previously been described for grassy stunt (11,13,16). Mixed infections by two or more viruses often induce more severe symptoms than does infection by a single virus. However, it is unlikely that GSW is caused by mixed infections by RGSV and RRSV because GSW symptoms have occurred in rice fields where ragged stunt was absent or rare. According to Ling et al (14) and Hibino et al (8), symptoms of both diseases were expressed in dual RGSV and RRSV infections. We have never observed the characteristic ragged stunt symptoms, such as ragged leaves and vein galls, in experimentally GSW-diseased plants.

The average incubation periods of the GSW, GSB, and GSY agents in *N. lugens* agree fairly well with the incubation period of 9.5–10.6 days reported for RGSV (11,16).

Cellular inclusions in the form of bundles and fibrillar structures 1,500 nm in length were recently found in rice plants affected with GSW (3,4). These inclusions closely resemble those found in rice plants affected with grassy stunt (15). In both cases, the inclusions were observed in both the nucleus and the cytoplasm. This is an additional basis for grouping the two diseases together, although the nature of the inclusions has yet to be determined. More reliable

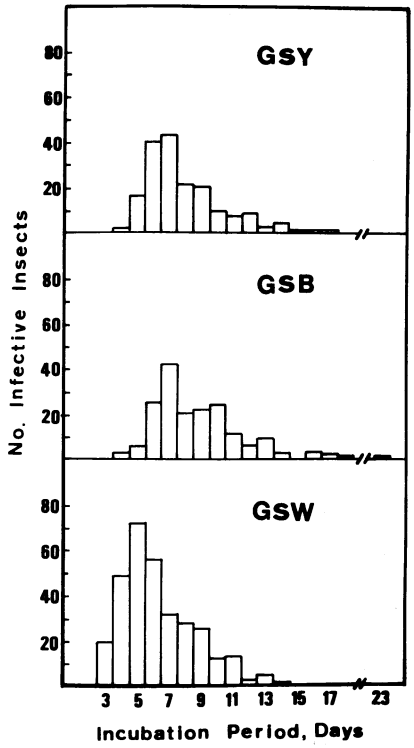

Fig. 2. Incubation periods of agents of three diseases related to grassy stunt in the rice brown planthopper *Nilaparvata lugens*. Data representing 176, 175, and 323 observations (insects) for grassy stunt Y (GSY), grassy stunt B (GSB), and wilted stunt (GSW), respectively, were accumulated from 10 to 14 separate tests over the 8-mo period of April–October 1979.

identifications could be based upon virus particle morphology, and the recent demonstration of 20-nm spherical particles for RGSV (17) will undoubtedly encourage future studies on this group of rice viruses transmitted by brown planthoppers.

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