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## Spread and yield loss mechanisms of rice stripe disease in rice paddies

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### ABSTRACT

Rice stripe disease is an economically important disease of rice caused by the *Rice stripe virus* (RSV), which is transferred by the small brown planthopper (SBPH). The recent rapid increase in damage to rice crops throughout Japan caused by this disease makes it imperative to develop control methods as soon as possible. To obtain basic data for developing such methods, we studied how the disease causes damage and spreads within paddy fields. Our investigations revealed that diseased plants first appear in mid-June to early July, after which the disease spreads from affected plants to adjacent plants. This suggests that SBPH carrying RSV enter paddy fields, where they infect plants as they move about and lay eggs. Subsequently, hatched viruliferous nymphs infect surrounding plants, thereby spreading the disease. Our analysis of the damage caused by rice stripe disease showed that the earlier the onset of disease, the more extensive the damage caused, and that the disease reduces yield by reducing the number of healthy panicles. This suggests that to reduce damage caused by this disease, it is necessary to ensure the growth of a sufficient number of healthy panicles by controlling the vector insect during the crop's early growth period. To be most effective, pest control efforts should be timed to target either the first-generation adults that colonize the paddy fields or the second-generation nymphs and adults that cause the rapid increase in the number of diseased plants within a field.

### 1. Introduction

Rice stripe disease is one of the most serious viral diseases affecting rice (*Oryza sativa* L.) crops in Japan, South Korea, and China. The disease is caused by the *rice stripe virus* (RSV, Toriyama, 1983), in the genus *Tenuivirus* (Shirako et al., 2011), which is persistently transmitted by the small brown planthopper (SBPH, *Laodelphax striatellus* (Fallén)) and is passed to the next generation by transovarial transmission (Hibino, 1996; Toriyama, 1983). In Japan, RSV caused widespread damage from the 1960s to the 1980s, but was brought under control from the late 1980s through control of the vector insect, increased use of RSV-resistant rice cultivars, and other measures (Hibino 1996). However, in recent years, rice stripe disease has returned with a vengeance in the Kanto region (the east-central area of Japan's main island), the Kinki region (the west-central area of Japan's main island), and the Kyushu region (southwestern Japan) (Shiba et al., 2016; Yoshida et al., 2014). Serious outbreaks have also been reported in China and South Korea (Jonson et al., 2009; Wang et al., 2008). It is not yet known why this disease has re-emerged in East Asia, but suspected

causes include the development of pesticide resistance by SBPH (Sanada-Morimura et al., 2011), climate change (Yamamura and Yokozawa, 2002), mass immigration of SBPH from overseas (Otuka et al., 2010, 2012), and changes in the cropping systems and environments surrounding production areas.

Susceptibility to RSV in rice varies widely with growth stage (Adachi and Yamada, 1968; Hibino, 1996; Wang et al., 2008). Rice in the early vegetative phase (from planting to the early tillering stage) is highly susceptible to RSV. Leaves of tillers infected during this period develop a mosaic of light yellow or yellow-green lesions along their veins, and new leaves curl and droop instead of fanning out. The majority of tillers that show these symptoms wilt without heading. In the late vegetative phase (the late tillering stage), susceptibility to RSV declines, and wilting due to infection does not occur. However, infected tillers cannot head normally; instead, they produce deformed panicles. Plants in the reproductive phase following panicle initiation are less susceptible to infection, and even if they are infected, symptoms are not severe.

The typical SBPH life cycle in areas of Japan prone to rice stripe

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disease is described by Shiba et al. (2016). Nymphs overwinter in patches of grass, and adults of the overwintering generation emerge in spring and move to adjacent wheat fields to propagate. Adults of the next generation (first generation) colonize paddy fields after rice seedlings have been planted. After three or four generations in the paddy fields, adults move to nearby grassy areas during the harvest season to lay eggs, and the next generation overwinters as nymphs. Because wheat is an ideal SBPH food source, SBPH numbers are liable to increase in areas where wheat is grown, and rice stripe disease tends to occur more frequently in these areas.

Research on the epidemiology and control of rice stripe disease in Japan was carried out intensively from the 1960s to the 1980s, but since then, factors that affect rice stripe disease epidemiology such as the cultivar, cropping system, and surrounding environment have changed substantially, rendering much of the knowledge gained in that period inapplicable. With rice stripe disease once more becoming pervasive in Japan, we launched a comprehensive research project to develop control techniques aimed at early containment of outbreaks. We have previously reported that measurements of the effective cumulative temperature can be used to accurately predict the appearance of SBPH in paddy fields (Hirae and Shiba, 2016), and that the elimination of rice ratoons and of grass near paddies after harvest is critical to suppressing the disease (Shiba et al., 2016). Here, we report on the mechanism by which rice stripe disease causes damage to infected rice plants, and how the disease spreads through paddy fields. This is essential information to developing effective control techniques against the current outbreak of rice stripe disease.

## 2. Materials and methods

### 2.1. Test plots

From 2012–2014, we conducted experiments in Nikinari, a district of Chikusei City, Ibaraki Prefecture, in Japan's Kanto region (36°17'N, 139°58'E), where rice stripe disease occurs every year. We planted seedlings of 'Koshihikari' (which is susceptible to RSV), Japan's most widely grown cultivar of rice, in two paddy fields in each year. In 2012, Fields A and B each covered approximately 3000 m<sup>2</sup> and were 65 m apart at their closest points. In 2013, Fields C and D each covered approximately 7000 m<sup>2</sup> and were 60 m apart at their closest points. In 2014, Fields E and F each covered approximately 3000 m<sup>2</sup> and were 100 m apart at their closest points. The seedlings were planted 24 cm apart in rows 30 cm apart. Each field was planted in mid-May (15 May 2012, 17 May 2013, 14 May 2014) and harvested in early to mid-September (12 September 2012, 18 September 2013, 9 September 2014). No pesticides were applied during cultivation in each of the test plots. In 2012, we established rectangular plots of 30 rows with 73 plants per row in each field, and also selected individual plants within each plot for detailed observation. Every fifth plant in every third row was designated as a fixed-point-survey plant, for a total of 15 such plants per row in 10 rows. Two of those plants in Field A failed to survive. Thus, the fixed-point-survey for Field A included only 148 plants, compared with 150 in Field B. In the same manner, we established rectangular plots of 30 rows with 50 plants per row in each field and designated 99 or 100 fixed-point-survey plants within each plot in 2013 and 2014.

In the experimental area, first-generation SBPH adults colonized the survey fields in mid-June, second-generation nymphs emerged in the paddy fields from late June to early July, and third-generation nymphs emerged from late July to early August according to estimates based on the measurements of the effective cumulative temperature obtained from JPP-NET (Japan Plant Protection Association, Tokyo, Japan). Rates of virus-infected first-generation adults of SBPH collected in rice paddies were 3.2% in 2012 (Shiba et al., 2016), 4.7% in 2013 (Shiba et al., 2016), and 16.8% in 2014 (Ibaraki Control Station for Pests, 2014).

### 2.2. Disease surveys

In 2012, we investigated all plants in the survey plot in Field A to detect the presence of diseased plants on 11 July (the panicle initiation stage), on 8 and 9 August (the flowering stage), and on 4 and 5 September (immediately before harvest). In addition, on the fixed-point-survey plants, we counted the numbers of total, diseased, and healthy panicles during the survey in early August. In Field B, we investigated disease incidence among the fixed-point-survey plants and the surrounding 8 plants on the same dates as the Field A surveys. As in Field A, we also counted the number of total, diseased, and healthy panicles of the fixed-point-survey plants in Field B in early August. We judged plants to be diseased if they showed typical rice stripe disease symptoms, such as wilted new leaves, mottled leaves, or deformed panicles. We categorized diseased plants identified during the early July survey as "mid-June to early-July onset" plants, those newly identified during the early-August survey as "mid-July to early-August onset" plants, and those newly identified during the early September survey as "mid-August to early-September onset" plants. Because the area chosen for this study is almost entirely free of pests and diseases other than rice stripe disease, we ignored the presence of other pests and diseases. In the same manner as in 2012, we investigated disease incidence on the fixed-point-survey plants in 2013 and 2014. Surveys were conducted on 11 and 12 July, 8 and 9 August, and 29 August 2013, and on 10 July, 7 and 8 August, and 28 August 2014.

### 2.3. Yield survey

In 2012, we harvested all fixed-point-survey plants that developed rice stripe disease up to harvest time, and evaluated the number of total, healthy, and diseased panicles, the brown rice yield, the number of brown rice kernels, and the 1000-kernel weight of each plant. We also randomly harvested half of the disease-free fixed-point-survey plants in each plot and evaluated yield in the same manner. In cases in which a fixed-point-survey plant was unlikely to yield a large enough sample for analysis, we also harvested surrounding plants. The above measurements were taken after harvesting individual plants from the survey fields and drying them naturally for a month inside field cages. In conformity with Japanese survey standards for paddy rice yield (Hosaka, 2014), any brown rice grains with a diameter of  $\leq 1.69$  mm were excluded from the survey.

### 2.4. Statistical analysis

We conducted two-way ANOVA for brown rice yield, total number of panicles, the number of healthy panicles, and 1000-kernel weight by survey field, disease onset period, and their interaction. When two-way ANOVA showed the disease onset period to have a significant effect, we performed the Tukey–Kramer HSD test as a *post-hoc* test. To analyze the relationship between the number of healthy panicles and brown rice yield, we conducted simple regression analysis of yield on the number of healthy panicles for each disease onset period. We used Pearson's correlation coefficient to analyze the relationship between the number of panicles at the flowering stage and at harvest, and conducted paired *t*-tests to confirm that the difference in the number between flowering and harvest was significant. To investigate how the disease spreads, we performed spatial autocorrelation analysis using join-count statistics (Cliff and Ord, 1981; Plant, 2012) on the data from the 30-row  $\times$  73-plants-per-row survey plot in Field A, in which all plants were checked for disease. We used the *spdep* package (Bivand et al., 2013) for version 3.3.3 of the R statistical software (R Core Team, 2017) for the join-count statistical analyses, and version 12.2.0 of the JMP software (SAS Institute, Cary, NC, USA) for the other analyses.

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