

Induced systemic resistance to rice blast fungus in rice plants infested by white-backed planthopper

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Introduction

Many of the studies on herbivorous insects and also on pathogens have been conducted to clarify the relationship of insects to plants and of pathogens to plants. Studies on the relationship between insects and pathogens via host plants, however, have seldom been conducted (Karban et al., 1987).

The white-backed planthopper, *Sogatella furcifera* Horvas (Homoptera: Delphacidae), and the rice blast disease caused by *Pyricularia grisea* Cooke, are an economically important insect pest and fungal disease of rice throughout south-east and far-east Asia, including Japan. In general, *S. furcifera* does not hibernate in Japan. The entire original *S. furcifera* population emigrates from mainland China into Japan during the rainy season from early June to early July. The population of the next generation of *S. furcifera* then increases rapidly in rice fields, and the highest peak of the population appears around late July or early August (Watanabe et al., 1991; Watanabe, 1996). At the same time, the rice blast disease caused by *P. grisea* develops commonly in rice fields in Japan and occasionally causes serious damage to the rice plants (Iwano, 1999). The authors therefore were interested in the interspecific relationships between *S. furcifera* and *P. grisea* via the host plants. In this paper, the results of experiments elucidating such interspecific relationships are discussed, in particular the effect of *S. furcifera* infestation in rice on the incidence of rice blast disease caused by *P. grisea*.

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Materials and methods

Test plants

The Japonica rice variety Hinohikari, containing blast-resistant genes Pi-a and Pi-i (Iwano, 1999), was used in all experiments. Plants were grown under glasshouse conditions (25 °C ± 1 °C, 60–80% r.h.) from seeds planted in plastic pots containing a commercial potting soil, Hinokuni-shodo (Japan Agricultural Co.), mixed with fertilizers (nitrogen: 0.02%, phosphoric acid: 0.05%, potassium: 0.04%). Tests were conducted on plants grown to around the five-leaf stage, about 4 weeks post seeding.

Test insects

Sogatella furcifera was obtained from a laboratory-reared culture originating from adults collected in 1990 from a rice field in Chikugo, Fukuoka Prefecture, Japan. The Japonica rice variety 'Reihoh' susceptible to *S. furcifera*, was used for insect rearing. Adults of *S. furcifera* were allowed to oviposit on rice plants grown to the two-leaf stage in an insect rearing cage (25 cm × 30 cm × 25 cm). The rearing cages contained approximately 1000 planthoppers, and they were placed in a controlled environment chamber (25 °C, 50–70% r.h., L14:D10) until adults emerged 20 days after oviposition. These adults were used for all experiments.

Test fungal inoculums

The colony of *Pyricularia grisea* (race number: 007) used for this experiment originated in 1995 from a diseased rice paddy in Nishigoshi, Kumamoto Prefecture, Japan. The colony was incubated on oatmeal sucrose agar (OMSA) at 25 °C in the dark for 2 weeks. The OMSA plates were then flooded with a 0.02% Tween 20 (surfactant) solution. The conidial suspension of *P. grisea* was prepared by softly scraping the mycelia from the surface of the plates using a paintbrush. A suitable concentration of conidia in distilled

water (about 500 per mm²) was carefully determined by microscopic observation at a magnification of $\times 150$.

Test 1. Effect of *S. furcifera* infestation on the incidence of rice blast disease

Test 1 was conducted to verify that infestations of *S. furcifera* induce resistance to blast disease in rice. A cage (50 \times 50 \times 50 cm, plastic rod frame covered with a fitted cotton mesh net) containing ten rice plants at the five-leaf stage with 100 pairs of newly emerged adults (100 males and 100 females) was used for *S. furcifera* infestation. As a control, ten plants were placed in a second cage with no additional insects. Twenty-four hours later, all treated and control plants were removed from the cage, and sprayed with 2 ml per plant of conidial suspension using an airbrush sprayer. Plants were incubated in a dew chamber (25 °C, 100% r.h.) for 18 h before transferring to the glasshouse. The number of typical blast lesions, called S-lesion (susceptible lesion), on the plants was counted 7 days post inoculation. The experiment was replicated three times. The data were analyzed by Tukey–Kramer test (SAS, 2000).

Test 2. Effect of infestation by each sex of *S. furcifera* on the incidence of the rice blast disease

Test 2 was designed to determine if the induced resistance to blast in rice by *S. furcifera* was dependent on the sex of the insects. Sixty rice plants at the five-leaf stage encased in transparent plastic cylinders (15 cm in diameter and 70 cm in height) were divided into three equal groups. The groups of 20 plants each were used individually for three categories: (i) male adult infestation, (ii) female adult infestation, and (iii) uninfested control. In the case of the first two categories, ten male adults or ten female adults were released into a cylinder, and allowed to feed and oviposit on the plant. Twenty-four hours later, all of the test plants were inoculated with *P. grisea* and incubated as described in Test 1. After 7 days, the number of S-lesions of rice blast on the plants was counted. The data were analyzed by Tukey–Kramer test (SAS, 2000).

Test 3. Effect of restricted infestation of *S. furcifera* on the incidence of rice blast disease

Test 3 was conducted to confirm whether or not the induced resistance observed in Test 1 and 2 is a systemic phenomenon. Forty rice plants at the five-leaf stage were prepared and divided into two groups, *S. furcifera*-infested plants and uninfested ones. In this experiment, *S. furcifera* infestation was restricted to the stems of the test plants, which were encased in transparent plastic cylinders (5 cm in diameter and 15 cm in height). Five pairs of *S. furcifera* were released into each cylinder in the infested group. Twenty-four hours later, all test plants were inoculated

with *P. grisea* and incubated as described in Test 1. The number of blast S-lesions on the plants was counted 7 days after inoculation. The data were analyzed by Tukey–Kramer test (SAS, 2000).

Results and discussion

In test 1, rice plants that had previously been exposed to *S. furcifera* were less likely than controls to develop blast lesions caused by *P. grisea*. The number of blast lesions, especially S-lesions, on the leaves of rice plants that were infested by *S. furcifera* was significantly lower at $P < 0.05$ than on the uninfested plants (Figure 1). In the second test, blast incidence was strongly suppressed in both plant groups, infested with male adults or female adults of *S. furcifera*. There was no significant difference at $P < 0.05$ in blast damage between the two sexes (Figure 2). In the third test, there was a significant difference at $P < 0.05$ on the number of blast lesions between the two groups, *S. furcifera* infested and uninfested plants, when the infestation was restricted only to the rice plant stems. The number of blast lesions on the plants that were previously exposed to *S. furcifera* was around 40% lower than that of uninfested control plants (Figure 3).

These results show that *S. furcifera* infestation can strongly and negatively affect the development of rice blast symptoms. *Sogatella furcifera* was no longer present in the first experiment when rice plants were inoculated with conidia, therefore infestation of *S. furcifera* must have

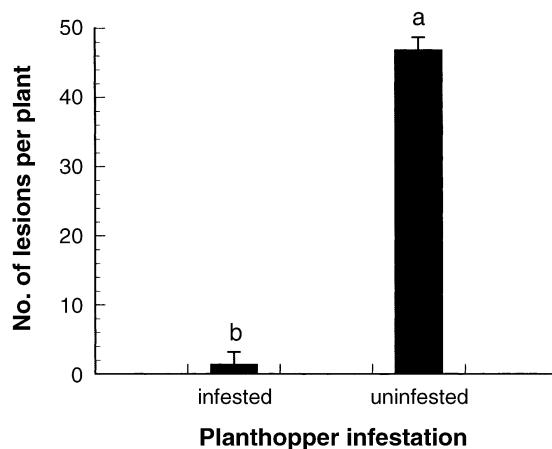


Figure 1 Incidence of rice blast disease caused by *Pyricularia grisea* on rice (Japonica variety: Hinohikari) infested previously by adults of the white-backed planthopper, *Sogatella furcifera*. Standard bars (SB) indicate mean number of blast lesions per plant, and vertical bars on the SB indicate SE. Means accompanied by different letters are significantly different at $P < 0.05$ (Tukey–Kramer test; SAS, 2000).

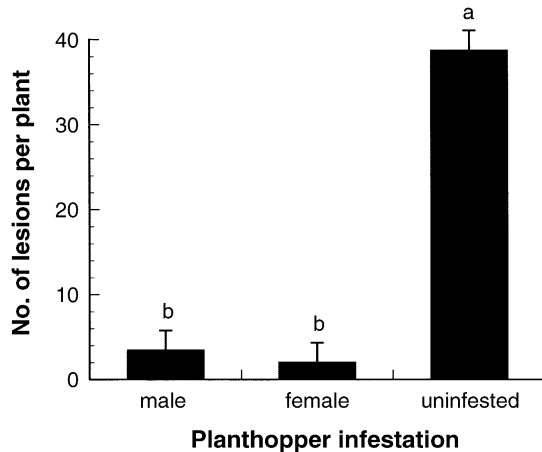


Figure 2 Incidence of rice blast disease caused by *Pyricularia grisea* on rice (Japonica variety: Hino hikari) infested previously by male adults or female adults of the white-backed planthopper, *Sogatella furcifera*. Standard bars (SB) indicate mean number of blast lesions per plant, and vertical bars on the SB indicate SE. Means accompanied by different letters are significantly different at $P < 0.05$ (Tukey–Kramer test; SAS, 2000).

induced physiological changes in the plant that reduced its susceptibility to the fungus, *P. grisea*. The incidence of blast disease did not significantly differ in the presence of either all-male or all-female *S. furcifera*, thus we hypothesize that the lower disease incidence was induced by the feeding behavior of either sex rather than the oviposition behavior of females. The incidence of rice blast is also suppressed by

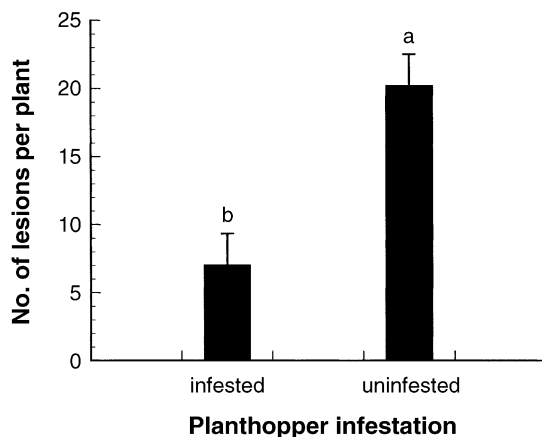


Figure 3 Incidence of rice blast disease caused by *Pyricularia grisea* on rice (Japonica variety: Hino hikari) infested previously on the stems by the adults of the white-backed planthopper, *Sogatella furcifera*. Standard bars (SB) indicate mean number of blast lesions per plant, and vertical bars on the SB indicate SE. Means accompanied by different letters are significantly different at $P < 0.05$ (Tukey–Kramer test; SAS, 2000).

restricted infestation of *S. furcifera* to only the stems of the host plant. These results indicate that induced physiological changes in the plants, reducing susceptibility to *P. grisea*, are a systemic phenomenon.

The interspecific relationship between two kinds of insects has been studied in several insect species. Attacks by herbivorous insects induce chemical and physical changes in many host plants (Green & Ryan, 1972; Tallamy & Raupp, 1991; Baldwin, 1994; Dicke, 1994; Schoonhoven et al., 1998). In several cases, host changes caused by herbivore damage have had deleterious effects on subsequent herbivores. Herbivores feeding on damaged plant tissue had lower survival rates, reduced individual growth rates, and reduced adult weight or fecundity, or both (Karban & Myers, 1989; Denno et al., 1995). The interspecific relationship between insects and pathogens, however, has seldom been studied. The negative interspecific effects between a herbivore and a fungus have only been observed in the spider mite, *Tetranychus urticae* Koch, and vascular wilt fungus, *Verticillium dahliae* Klebahn (Karban et al., 1987). It was demonstrated that changes in cotton seedlings caused by previous exposure to *T. urticae* reduced the probability of infection and severity of symptoms caused by *V. dahliae*.

Several mechanisms could produce a resistance pattern. Physiological changes in the host, which require no mechanism of recognition or response to a unique component of the attacker, may reflect wounding or deterioration of metabolic functions. An attacker might deplete nutrients or consume plant parts. Alternatively, resistance may be caused by metabolites that undergo activation or de novo synthesis and accumulate in plants after attacks. Chemicals that accumulate after damage may affect a diverse range of organisms. For example, phytoalexin isoflavonoids reduce fungal growth and deter herbivorous insect feeding (Karban et al., 1987).

Recently, the mechanism of induced resistance in plants has been clarified at the molecular level. For example, in the tomato plant, it has been demonstrated that mechanical wounding, herbivore attack, and pathogen attack can elicit a defense response, such as the jasmonic acid pathway and/or the salicylic acid pathway. Then some phytoalexins and pathogenesis related proteins (PR-proteins) against pathogens and herbivores are finally induced in the plants (Fidantsef et al., 1999; Sivasankar et al., 2000; Orozco-Cardenas et al., 2001). In rice, Schweizer et al. (1997) confirmed that some PR-proteins are produced after activation of the jasmonic pathway by pathogen attack.

Our results indicate that some physiological changes may occur in rice when fed upon by *S. furcifera*. This insect generally infests the stems and occasionally the leaves of rice plants, and imbibes phloem sap. The authors hypothesize

three possible linked mechanisms of the phenomenon, viz., (i) the saliva of *S. furcifera* may contain an elicitor, (ii) the defense systems, such as jasmonic acid pathway and/or salicylic acid pathway, may be activated by the elicitor introduced into vascular bundles, and (iii) the phytoalexins and PR-proteins that act against pathogens might be produced in the rice plants. These hypotheses of the mechanism will be examined in the next step in this research series.

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