Preinfestation with the whitebacked planthopper (WBPH), *Sogatella furcifera* Horváth, conferred resistance to rice blast (RB) caused by *Magnaporthe grisea* in rice under both laboratory and field conditions. Infestation with either male or female WBPH induced resistance, indicating that their feeding behavior was responsible for the resistance. WBPH infestation restricted to the leaf sheaths also induced resistance, suggesting that the resistance is a systemic phenomenon. Simple mechanical wounding of rice plants did not induce resistance. WBPH infestation induced the expression of pathogen-responsive genes (*PBZ1*, *POX22.3*, *OsPR1a*, *OsPrb1*, and *OsWRKY70*); expression of these genes is normally induced after inoculation with avirulent *M. grisea* and/or *Xanthomonas oryzae* pv. *oryzae* (the pathogen causing bacterial blight of rice). We speculate that these genes play a role in increasing the ability of WBPH-infested rice to respond rapidly and effectively to subsequent pathogen attack.

**Keywords**: *Sogatella furcifera*, *Magnaporthe grisea*, induced resistance, *PBZ1*, *POX22.3*, *OsPR1a*, *OsPrb1*, *OsWRKY67*, *OsWRKY70*

Attacks by herbivorous insects induce chemical and physical changes in many host plants (Karban and Baldwin 1997, Dicke 1994). In several systems, host changes caused by herbivore damage have had deleterious effects on herbivores making subsequent host attacks. Herbivores that feed on damaged plant tissues have lower survival rates, reduced individual growth rates, and reduced adult weight or fecundity, or all three (Karban and Myers 1989, Denno et al 1995). Matsumura and Suzuki (2003) reported that infestation of rice plants with the whitebacked planthopper (WBPH) *Sogatella furcifera* Horváth (Homoptera: Delphacidae) induced resistance against subsequent infestation with WBPH and the brown planthopper (BPH) *Nilaparvata lugens*; that is, there was a deterioration in the performance and promotion of flight-capable adults that could disperse. However, information about the interspecific relationship between insects and pathogens is limited (Stout et al 2006).

In rice, WBPH infestation and rice blast (RB) disease caused by *Magnaporthe grisea* are economically important throughout Southeast and Far-East Asia, including
Japan. In general, WBPH does not hibernate in Japan. The entire WBPH population emigrates from mainland China to Japan during the rainy season from early June to early July. There, the population of the next generation increases rapidly in rice fields, and its peak occurs in about late July or early August (Watanabe et al 1991). At the same time, RB caused by \textit{M. grisea} also commonly develops in the rice fields of Japan. In addition, Kashiwagi and Nagai (1975) found a correlation between the occurrence of WBPH and RB. We therefore evaluated the interspecific relationships between WBPH and \textit{M. grisea} through the host plants.

Materials and methods

\textbf{Plant and insect materials}

Rice plants (\textit{Oryza sativa} L. cv. Hinohikari) were grown from seed under glasshouse conditions (25 ± 1 °C, 60–80% relative humidity) and used in all experiments. Tests were conducted on plants grown to around the 5-leaf stage (about 4 weeks postseed-ing). WBPHs were obtained from a laboratory-reared culture originating from adults collected in 1990 from a rice field in Chikugo, Fukuoka Prefecture, Japan.

\textbf{Fungal inoculation}

\textit{Magnaporthe grisea} race 007, which was compatible with Hinohikari, was grown on oatmeal medium for 2 weeks at 26 °C in the dark, and spor formation was induced by placing the cultures under a 20-W BLB light for 2 to 3 days at 24 °C. Approximately 2 mL per plant of a spore suspension (3 \times 10^5 conidia mL^{-1}) containing 0.05\% Tween-20 was sprayed onto the rice plants. The inoculated plants were incubated at 25 °C with high humidity in the dark for 20 h, and then moved to a greenhouse (25 °C). The number of typical blast lesions, called S-lesions (susceptible lesions), on the plants was counted 7 days after inoculation.

\textbf{Effect of WBPH infestation on RB incidence}

To verify that infestation with WBPH induces resistance to RB in rice plants, we conducted an inoculation trial (Kanno and Fujita 2003). A cage (50 × 50 × 50 cm; plastic-rod frame covered with a fitted cotton-mesh net) containing 10 rice plants with 100 pairs of newly emerged WBPH adults was used for WBPH infestation. As a control, 10 plants were placed in another cage with no added insects. Twenty-four hours later, the cages and WBPHs were removed from the plants, and all the plants were then inoculated with \textit{M. grisea} as described above. Seven days after inoculation, the number of S-lesions on the plants was counted. The experiment was performed three times. The data were analyzed by Student’s \textit{t}-test.

\textbf{Effect of infestation with sex-segregated WBPHs on RB incidence}

To determine whether the decrease in the number of blast lesions induced by infestation with WBPH was dependent on the sex of the insects, we conducted a sex-segregated infestation test (Kanno and Fujita 2003). Sixty rice plants were encased in transparent plastic cylinders (15 cm in diameter and 70 cm in height) and divided into three equal
groups containing 20 plants each. One group of plants was infested with adult male WBPHs, the second was infested with adult female WBPHs, and the third group served as the uninfested control. In the case of the first two categories, 20 male adult WBPHs or 20 female adults were released into the cylinder and allowed to feed and lay eggs on the plants. Twenty-four hours later, the cylinders and WBPHs were removed from the plants, and all the plants were then inoculated with *M. grisea* as described above. Seven days after inoculation, the number of S-lesions on the plants was counted. The data were analyzed by the Tukey-Kramer test.

**Effect of restricted infestation with WBPHs on RB incidence**

We conducted this test to determine whether or not the observed resistance to RB induced by WBPH feeding was a systemic phenomenon (Kanno and Fujita 2003). In this experiment, to restrict WBPH infestation to the leaf sheaths, rice plants were encased in transparent plastic cylinders (5 cm in diameter and 15 cm in height) that covered only the leaf sheath region, and 5 pairs of adult WBPHs per plant were released into each cylinder. The leaf sheaths of control plants were covered by transparent plastic cylinders without planthoppers. Each group contained 20 plants. Twenty-four hours later, the cylinders and WBPHs were removed from plants, and all the plants were then inoculated with *M. grisea* as described above. Seven days after inoculation, the number of S-lesions on the leaves was counted. Data were analyzed by Student’s *t*-test.

**Effect of simple mechanical damage on RB incidence**

This test was conducted to investigate the effect of the mechanical simulation of WBPH feeding on the incidence of blast lesions (Kanno et al 2005). The leaves of 10 plants were mechanically wounded by puncture with a needle. The plant was punctured 50 times every 12 h for 48 h, so that the total number of needle punctures was 250 per plant. Another 10 plants were used as untreated controls. All the plants were then inoculated with *M. grisea* as described above. Seven days after inoculation, the number of S-lesions on the leaves was counted. Data were analyzed by Student’s *t*-test.

**Effect of WBPH infestation on RB incidence in the field**

To extend the laboratory characterization of WBPH-induced resistance to RB, we evaluated the resistance induced under field conditions (Satoh et al 2005). A field test was conducted in paddy fields at the National Agricultural Research Center for Kyushu Okinawa Region, at Koshi in Kumamoto Prefecture, during the summers of 2002 and 2003. Two adjoining paddy fields (each 10 × 50 m) were used in this test. Four-week-old rice seedlings, raised in nursery boxes, were transplanted (two or three seedlings per hill) in a field in late June.

In the first experiment, we conducted an inoculation trial in a field to which we had applied pesticide to suppress WBPH. In 2002, just before transplanting, the planthopper pesticide fipronil was applied (50 g per nursery box) to half of the seedlings, which were then transplanted in a paddy field (field A). Untreated seedlings were transplanted in another paddy field (field B). To verify the effect of fipronil, population surveys of planthoppers were conducted twice according to Nagata and
Masuda (1978). Subsequently, 6 rice hills per paddy field were inoculated twice with *M. grisea* with the inoculation method using clear cover materials (Kobayashi et al 2001). Seven days after inoculation, the number of S-lesions on the leaves was counted. Data were analyzed by two-way analysis of variance (ANOVA). In 2003, each field was divided into two divisions (5 × 50 m each) with wavy plastic boards to prevent water from traversing the divisions. Just before transplanting, the planthopper and leafhopper pesticide imidacloprid was applied (50 g per nursery box) to half of the seedlings, which were transplanted into two of the four divisions. Pesticide-treated and untreated seedlings were transplanted in the other divisions. Population surveys of planthoppers and leafhoppers were also conducted twice. Eight rice hills in each division were then inoculated with *M. grisea*, as described above. Seven days after inoculation, the number of S-lesions on the leaves was counted. Data were analyzed by two-way ANOVA.

In the second experiment conducted in 2002, six clusters of rice hills (each cluster consisting of 6 hills) that had not been treated with insecticide were randomly selected. Each rice hill was individually encased in a transparent plastic cylinder (60 cm high, 9.5 cm in diameter, top of cylinder covered with gauze). They were divided into two equal groups: plants on which WBPH had and had not been released. Ten pairs of WBPHs were released into each of the 10 cylinders for the WBPH treatment. Two days later, all cylinders were removed and all test plants, both with and without WBPH, were inoculated with *M. grisea* by cluster, as described above. Seven days after inoculation, the number of S-lesions on the leaves was counted. Data were analyzed by Student’s *t*-test.

**Defense-related rice gene expression analysis in response to WBPH feeding**

We used quantitative RT-PCR to analyze the expression patterns of defense-related genes in rice plants in response to WBPH infestation. We selected six genes, including some that encode PR (pathogenesis-related) proteins: *PBZ1* (encoding an intracellular, probenazole-inducible protein, *PR-10*), *POX22.3* (encoding a Class III plant peroxidase), *OsPR1a* (encoding an acidic PR-1-type pathogenesis-related protein), *OsPrb1* (encoding a PR-1 type pathogenesis-related protein), *OsWRKY67*, and *OsWRKY70* (encoding transcription factors involved in the regulation of plant defense response pathways). Rice plants covered with transparent plastic cylinders (15 cm in diameter and 70 cm high) were infested with 20 adult male WBPHs. As mock-treated controls, other plants were put in transparent plastic cylinders but insects were not released on them. All the cylinders and WBPHs were removed from plants, and immediately the 4th and 5th leaves were frozen in liquid nitrogen at time points of 0, 6, 12, 24, and 48 h. Total RNA was extracted from the leaves after each treatment by using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Quantitative RT-PCR was performed using iQ SYBR Green Supermix (BioRad, Hercules, CA) in an iCycler (BioRad) according to the manufacturer’s instructions. Data were analyzed by the method of De Vos et al (2005), with minor modifications. Transcript levels of each gene were normalized by comparison with levels of *actin* transcript.
Normalized transcript levels of the genes analyzed in each treatment were compared with those of the respective mock-treated controls, and the fold changes in expression level were calculated. The corresponding accession numbers used for quantitative RT-PCR were AK060893 for actin, AK071613 for PBZ1, AK073202 for POX22.3, AJ278436 for OsPR1a, AK060057 for OsPrb1, AK066252 for OsWRKY67, and AK119867 for OsWRKY70.

Results and discussion

RB resistance induced by WBPH feeding on rice plants
Rice plants that had previously been exposed to WBPHs were less likely than controls to develop blast lesions caused by *M. grisea*. The number of blast lesions on leaves that had been infested with WBPH was significantly lower at $P < 0.05$ than that on the uninfested plants (Fig. 1A). In the second experiment with sex-segregated WBPH populations, blast incidence on plants that had been infested with either male or female WBPHs was strongly suppressed compared with that on uninfested control plants. The difference in the number of blast lesions between male- and female-infested plants was very small and not statistically significant ($P > 0.05$) (Fig.1B).

These results show that WBPH infestation inhibits incidence of RB. In the first experiment, after removal of the insects, the plants were inoculated with *M. grisea*;
therefore, WBPH infestation induced physiological changes in the rice plant that reduced its susceptibility to later RB infection. It is known that japonica rice cultivars respond sensitively against oviposition of WBPH and produce an ovicidal substance, benzyl benzoate (Seino et al 1996). Moreover, the fact that the numbers of blast lesions did not differ significantly in the presence of all-male or all-female WBPHs indicates that disease inhibition was induced by the feeding behavior of either sex rather than by the oviposition behavior of females.

**Systemic effect of WBPH-induced RB resistance**

In the typical phenomenon of induced resistance in plants, systemic acquired resistance (SAR), plants respond to local attack by pathogens with a *de novo* production of compounds to resist pathogens. Responses occur not only in the plant organ originally attacked (local response) but also in yet unaffected distant parts (systemic response).

When WBPH infestation was restricted to the leaf sheaths, the number of blast lesions on the leaves that had been infested with WBPH was significantly lower at $P < 0.05$ than that on the uninfested control plants (Fig. 2). The number of blast lesions on plants of which the leaf sheaths had been previously infested with WBPH was about 40% lower than that on control plants. Therefore, WBPH-induced resistance to RB appears to be a systemic phenomenon such as SAR.
The mean number of S-lesions on needle-punctured rice plants was only slightly lower than that on control plants. There was no significant difference ($P > 0.05$) in the incidence of RB between wounded plants treated by needling and untreated control plants (Fig. 3). This result suggests that the trigger for the induced resistance is not the simple mechanical effect of WBPH feeding behavior on the plants; instead, it must be some chemical substance or substances in the WBPH’s saliva.

**Lack of effect of simple mechanical damage on RB incidence**

The mean number of S-lesions on needle-punctured rice plants was only slightly lower than that on control plants. There was no significant difference ($P > 0.05$) in the incidence of RB between wounded plants treated by needling and untreated control plants (Fig. 3). This result suggests that the trigger for the induced resistance is not the simple mechanical effect of WBPH feeding behavior on the plants; instead, it must be some chemical substance or substances in the WBPH’s saliva.

**Efficacy of WBPH-induced RB resistance under field conditions**

We used fipronil, a planthopper pesticide, to suppress WBPH in 2002. The population density of WBPH was lower on plants treated with fipronil than on untreated plants (Table 1). When the plants were inoculated with *M. grisea*, the number of blast lesions on plants treated with fipronil (Field A) was significantly higher than that on plants not treated with fipronil (Field B). In Field A rice plants inoculated with *M. grisea* on 16 July, the mean number of blast lesions per plant was 29.2; in Field B plants it was 11.2 (ANOVA, $F = 21.6, P < 0.001$). In Field A rice plants inoculated with *M. grisea* on 23 July, the mean number of blast lesions per plant was 13.2; in Field B it was 9.8 (ANOVA, $F = 14.3, P < 0.01$) (Table 2). In 2003, we used imidacloprid, a
pesticide of planthoppers and leafhoppers, to suppress WBPH because small numbers of the green rice leafhopper *Nephotettix cincticeps* (Uhler) (Homoptera: Cicadellidae) had been found in population surveys conducted the previous year. The population density of WBPH was lower on plants treated with imidacloprid than on untreated plants (Table 3). When the plants were inoculated with *M. grisea*, the number of blast lesions on plants that had been treated with imidacloprid was significantly higher than that on plants not treated with pesticide (ANOVA, $F = 13.7, P < 0.001$). There was no significant difference in the number of blast lesions between replications (ANOVA, $F = 3.4, P > 0.05$) (Table 4). These results indicate that the incidence of blast lesions in the field was significantly higher on plants to which fipronil or imidacloprid had been applied than on those to which pesticides had not been applied. The cause of this

<table>
<thead>
<tr>
<th>Date</th>
<th>No. of adults per hill (mean ± SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Field A</td>
<td>Field B</td>
</tr>
<tr>
<td>27 June</td>
<td>0.01 ± 0.01</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>8 July</td>
<td>0.02 ± 0.01</td>
<td>0.3 ± 0.05</td>
</tr>
</tbody>
</table>

*a* Containing plants to which fipronil had been applied as pretransplant seedlings. *b* Containing plants that had not received fipronil as pretransplant seedlings. *c* Number of adults was transformed to $(X + 0.5)^{1/2}$ before analysis. Significance was tested by Student’s *t*-test. Source: Satoh et al (2005).

<table>
<thead>
<tr>
<th>Date of inoculation of <em>M. grisea</em></th>
<th>No. of blast lesions per hill (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Field A</td>
</tr>
<tr>
<td>16 July</td>
<td>29.2 ± 2.8</td>
</tr>
<tr>
<td>23 July</td>
<td>13.2 ± 2.0</td>
</tr>
</tbody>
</table>

*a* Containing plants to which fipronil had been applied as pretransplant seedlings. *b* Containing plants that had not received fipronil as pretransplant seedlings. Source: Satoh et al (2005).
difference is surely WBPH-induced RB resistance: we speculate that suppressing the occurrence of WBPH suppressed WBPH-induced RB resistance.

We also conducted WBPH-release experiments in the field. When WBPH was released and allowed to feed on rice plants, the blast lesions on plants on which WBPH had been released were significantly lower at \( P < 0.005 \) than those on plants not exposed to WBPH. WBPH-induced resistance to RB under field conditions with the release of WBPH reduced the number of blast lesions to 20% of that in the control (Fig. 4).

### Table 3. Effect of imidacloprid application to pretransplant seedlings on the population density of *Sogatella furcifera* in paddy fields in 2003.

<table>
<thead>
<tr>
<th>Date</th>
<th>No. of adults per hill (mean ± SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imidaclorid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Applied</td>
<td>Not applied</td>
</tr>
<tr>
<td>3 July</td>
<td>0.4 ± 0.05</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>10 July</td>
<td>0.06 ± 0.02</td>
<td>4.2 ± 0.2</td>
</tr>
</tbody>
</table>

*Number of adults was transformed to \((X + 0.5)^{1/2}\) before analysis. Significance was tested by Student’s \( t \)-test.


### Table 4. Incidence of rice blast disease caused by *Magnaporthe grisea* on rice plants in paddy fields where the occurrence of *Sogatella furcifera* was regulated by imidaclorid.

<table>
<thead>
<tr>
<th>Replication</th>
<th>No. of rice blast lesions per hill (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imidaclorid</td>
</tr>
<tr>
<td></td>
<td>Applied</td>
</tr>
<tr>
<td>Rep. 1</td>
<td>0.4 ± 0.05</td>
</tr>
<tr>
<td>Rep. 2</td>
<td>0.06 ± 0.02</td>
</tr>
</tbody>
</table>

Defense-related gene expression analysis in rice plants

We had already investigated the up-regulation of two β-1,3-glucanase genes (Gns4 and Gns5) in rice plants infested with WBPH and had confirmed that expression of both genes was up-regulated in WBPH-infested plants compared with that in uninfested controls. β-1,3-glucanase is a PR-2-type pathogenesis-related protein and is well known as a defense-related substance induced in plants against fungi (Kanno et al. 2005).

We also analyzed the expression patterns of six defense-related genes, *PBZ1*, *POX22.3*, *OsPR1a*, *OsPrb1*, *OsWRKY67*, and *OsWRKY70*, in rice plants infested with WBPH. Expression of *PBZ1* was induced 12 h after WBPH infestation, peaking 24 h after infestation. *POX22.3*, *OsPR1a*, and *OsWRKY70* expression was induced and it peaked 24 h after WBPH infestation. *OsPrb1* expression was induced at 12 h and stayed at the same level until 48 h after WBPH infestation. Expression of *OsWRKY67* decreased quickly to half the basal level 6 h after infestation but increased slightly thereafter (Fig. 5). These genes are well established as defense-related genes involved in responses to inoculation of avirulent *M. oryzae* and/or *Xanthomonas oryzae* pv. *oryzae* (the pathogen causing bacterial blight of rice) (Midoh and Iwata 1996, Chittoor et al. 1997, Kim et al. 2001, Ryu et al. 2006). Our results suggest a role for these induced genes in increasing the ability of WBPH-infested rice to respond rapidly and effectively to subsequent pathogen attacks.
Conclusions and perspectives

As mentioned above, we revealed that infestation with WBPH induces resistance to subsequent attack by *M. grisea* and planthoppers. These facts suggest that the phenomenon of WBPH-induced resistance is effective against other pathogens. Consistent with this suggestion, we found that WBPH infestation also inhibits the development of bacterial blight of rice, which is among the most serious rice plant diseases of most rice-growing countries (Gomi et al, unpublished data). Moreover, we found that the induced resistance to the bacterial pathogen in rice was strong with WBPH but not with BPH infestation (Gomi et al, unpublished data). From these results, we speculated that various defense-related substances were produced in rice plants as a result of WBPH infestation but not of BPH infestation. Thus, we also performed large-scale screening with a rice DNA microarray to investigate the molecular mechanisms involved in WBPH-induced resistance. Up-regulation of vast amounts of genes, including many defense-related genes, was caused by the feeding of WBPHs but not by BPH on rice (Gomi et al, unpublished data). This suggests that rice plants undergo dramatic physiological changes in response to the feeding of WBPHs. Studies of the specificity of

Fig. 5. Quantitative RT-PCR analysis of six genes in rice infested with whitebacked planthopper (WBPH), *Sogatella furcifera*. Expression of these genes is induced after inoculation with avirulent *Xanthomonas oryzae* pv. *oryzae* (Midoh and Iwata 1996, Chittoor et al 1997, Kim et al 2001, Ryu et al 2006) or avirulent *Magnaporthe oryzae* (Kim et al 2001) in rice. Fold induction of six genes at 6, 12, 24, and 48 hours after infestation of WBPH in rice is shown. Values are the means ± SD of four independent samples.
the defense-related substances produced will be needed if we are to paint the whole picture of the rice plant response to WBPH feeding.

References


Notes

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