

# Development of Fungicide Resistance in Rice Blast Fungus, *Pyricularia oryzae* Cavera

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Organomercury fungicides had been widely used for control of rice blast disease in Japan till 1965 but since 1969 they have been entirely substituted with several kinds of newly developed non-mercurial fungicides, i.e. antibiotics, organophosphorus compounds and organochlorine compounds.

Since these new fungicides are more selective in their action against organisms than the organomercurials, several problems have been pointed out that would be caused by the substitution of the fungicides; for instance, control of some diseases which had been prevented by application of organomercury fungicides increased difficulties in selection and application techniques of the new fungicides, and so forth.

Development of resistance of rice blast fungus, *Pyricularia oryzae* Cavara, to these non-mercurial fungicides is also one of the possible problems which are feared to be caused in the near future because their narrow selectivity in toxicity might fail to inhibit some mutants of the fungus.

## Development of fungicide resistance

Recently, occurrence of kasugamycin resistant rice blast fungus was reported in the rice field in the Shōnai district of Yamagata Prefecture<sup>2)</sup>. Developments of resistance of the fungi to antibiotics blasticidin S<sup>3),6)</sup> and kasugamycin<sup>4),6)</sup> have been found under laboratory condition by prolonged exposure of the

fungus to the antibiotics<sup>3),6)</sup> or by selection from a large number of fungal cells (spores) in a short period<sup>4),6)</sup>.

Generally speaking, the resistant clones obtained by the latter method are virulent to rice plants and show nearly the same properties as the parent wild type fungus, except sensitivity to the fungicides. By the latter method, resistant clones of the fungus to organophosphorus fungicides were also obtained, which are also virulent to rice plants<sup>6)</sup>.

These experimental results suggest that at least naturally occurring mutation is a factor

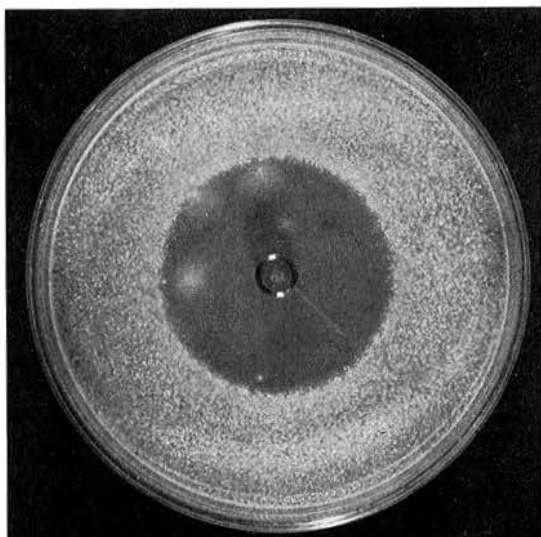


Fig. 1. Resistant clones occurred in inhibition zone by agar diffusion cup method using kasugamycin (1000 ppm) as test fungicide and an isolate of *P. oryzae* Ken-6491, as test fungus

of development of fungicide resistance, in other words, rice blast fungus occurring in the rice field always contains a very small number of the fungicide resistant mutants (Fig. 1). In this connection, an ecological problem remains to be solved—why the population of the resistant mutants stay at so low level until the fungicide is applied.

### Biochemical mechanism of fungicide resistance

There are many cases in which a microbe becomes resistant to a drug. Increased detoxification, decreased activation, decreased penetration through cell membrane of the drug and decreased sensitivity of the target enzyme to the drug have been proposed as factors for the drug resistance of the microbe.

Decreased penetration of blasticidin S through fungal cell membrane was proposed to be the resistance mechanism of the resistant rice blast fungus obtained by prolonged exposure to the antibiotic, because the protein synthesis, which is the site of fungicidal action of blasticidin S, was not inhibited when intact cells of the resistant fungi were used but was inhibited in the cell free system of the resistant fungus<sup>17</sup>.

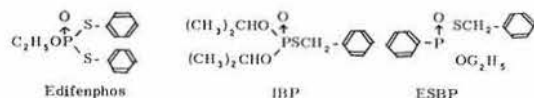
On the other hand, it was likely that kasugamycin resistance of rice blast fungus might be due to decreased sensitivity of the enzyme system.

Resistance to organophosphorus fungicides had been suspected to be due to their degradational detoxification on the analogy of organophosphorus insecticide resistance in insects which is, in most cases, related to metabolic detoxification.

Metabolism of the organophosphorus fungicides was, therefore, investigated by S<sup>35</sup>-, P<sup>32</sup>-, C<sup>14</sup>-labeled and S<sup>35</sup>-, P<sup>32</sup>-double labeled compounds comparing the resistant clone with its parent susceptible clone. No significant difference in metabolic pattern was found between the susceptible and the resistant clones both qualitatively and quantitatively in each case of edifenphos (EDDP, Hinosan®)<sup>9</sup>, IBP

(Kitazin P®)<sup>9</sup>, and ESBP (Inezin®)<sup>10</sup>.

Further investigation of resistance mechanism to organophosphorus fungicide is now in progress.



### Cross-resistance and other items important in preventing fungicide resistant fungi

When an organism resistant to a drug is also resistant to another drug, this resistance is called cross-resistance. It is important for the control of a resistant phytopathogenic microbe to a drug to examine its cross-resistance to other drugs.

Thus, a rice blast fungus resistant to an antibiotic has no cross-resistance to organophosphorus fungicides, and a fungus resistant to an organophosphorus fungicide has no cross-resistance to both the antibiotics but is cross-resistant to other organophosphorus fungicides having thiolate structure, >P(O)S<sup>-</sup>.

Further investigation for cross-resistance of phosphorothiolate resistant fungi to a wide range of chemicals, including organophosphorus compounds other than phosphorothiolates, led to a finding that the phosphorothiolate resistant fungi are specifically susceptible to some phosphoramidates, which have structure of >NP(O)<, though their thiolate susceptible parent clone is resistant to the amidates (Fig. 2)<sup>8</sup>. Such drug resistance (and related susceptibility), called negatively correlated cross-resistance, would become a powerful means in prevention of the drug resistant organism.

Since this negative correlation of cross-resistance between organophosphorus thiolates and amidates suggested some relationship in their fungicidal action, joint action of the amidates and the thiolates was investigated

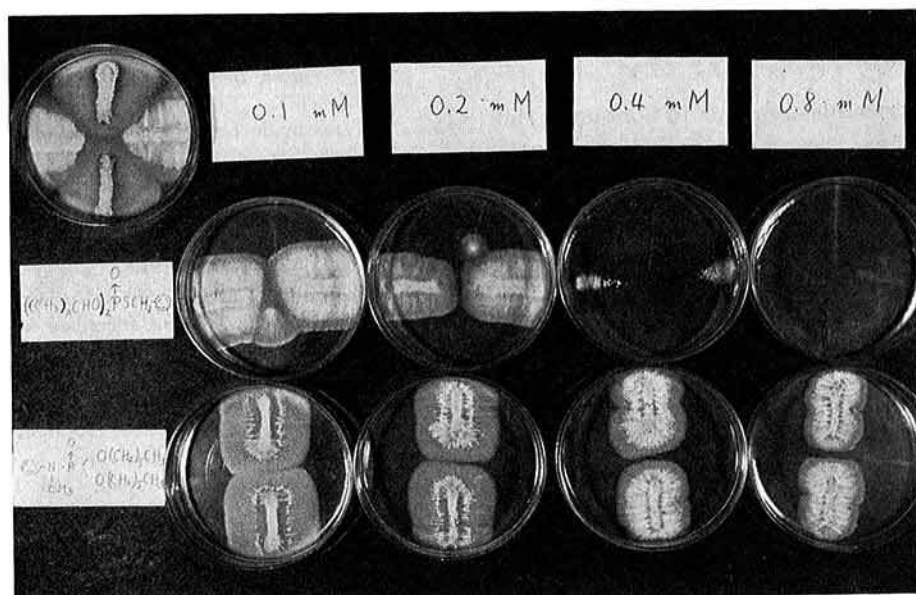


Fig. 2. Negatively correlated cross-resistance shown by growth of the wild type rice blast fungus (inoculated vertically on each plate) and the organophosphorus thiolate resistant clone (inoculated horizontally) on agar plates containing an organophosphorus thiolate fungicide (middle line) and an amidate (lower line), contrasting with that on fungicide untreated agar plate (upper left)

by crossing the paper strips each impregnated with respective fungicide on agar plate seeded uniformly with spores of test fungus.

Remarkable expansion of inhibition zone was observed at the crossing of the paper strip impregnated with the thiolates and that with the amidates when wild type (thiolate susceptible) rice blast fungus was used as test organism (Fig. 3), but it was not observed when the thiolate resistant clone was used<sup>9</sup>. This expansion of inhibition zone shows, of course synergism in fungicidal activity between organophosphorus thiolates and amidates.

Later work revealed that some acid amide compounds, for instance propanil (3', 4'-dichloropropionanilide) and its derivatives are also synergistic in fungicidal activity with phosphorothiolates on wild type rice blast fungus but not on phosphorothiolate resistant fungus<sup>7</sup>. In this case, however, negative correlation in cross-resistance with phosphorothiolate was not observed.

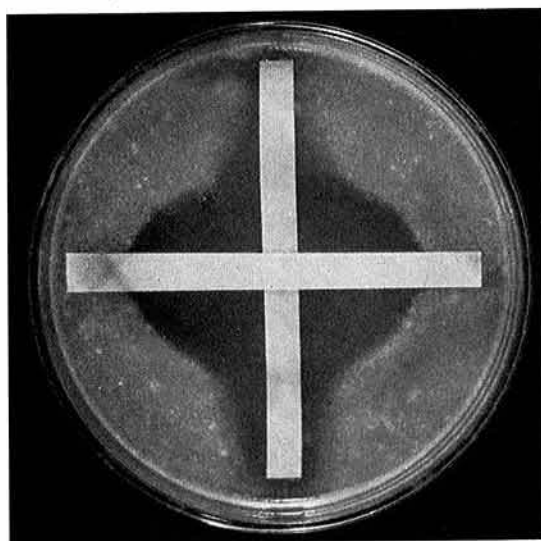


Fig. 3. Synergism of a phosphoramidate with an organophosphorus thiolate fungicide (IBP) shown by the pattern of inhibition zone around the crossed paper strips impregnated with the thiolate (vertical one) and the amidate (horizontal one) respectively on agar plate seeded uniformly with a wild type rice blast fungus

## Conclusion

Resistant rice blast fungus to non-mercurial fungicides has been investigated previously to its occurrence in the practical rice field. Some phosphoramidates are negatively correlated with organophosphorus thiolate fungicides in mode of cross-resistance of rice blast fungus, and it also showed synergism with the thiolates on wild type of rice blast fungus.

This finding would be a powerful measure not only in prevention of the drug resistant fungus in the practical field, but also in clarification of the resistance mechanism and the mode of action of the fungicides.

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