

## Similarities Between the Fungicidal Action of Isoprothiolane and Organophosphorus Thiolate Fungicides

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### ABSTRACT

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A similarity in the mode of action of isoprothiolane and organophosphorus thiolate (PTL) fungicides in *Pyricularia oryzae* is suggested because cross resistance was found between them, and synergistic joint action and negatively

correlated cross resistance also were found between isoprothiolane and phosphoramidate (PA) compounds as in the case of the PTL fungicides and PA compounds.

Rice blast caused by *Pyricularia oryzae* is an important disease in Japan. Several types of fungicides are used to control this disease. The organophosphorus thiolate (PTL) fungicides which includes compounds such as *S*-benzyl diisopropyl phosphorothiolate (IBP, Kitazin P, Fig. 3) and edifenphos (ethyl *S*, *S*-diphenyl phosphorodithiolate, EDDP, Hinosan) are one major group used for this purpose.

Recently another fungicide, isoprothiolane (diisopropyl 1,3-dithiolan-2-ylidenemalonate, Fuji-One, Fig. 3) was developed and brought into practical use for control of rice blast. This paper deals with the cross resistance of *P. oryzae* to PTL fungicides and isoprothiolane and with other similarities between these compounds such as negatively correlated cross resistance and synergism with *N*-alkyl-*N*-phenylphosphoramidate (PA) compounds.

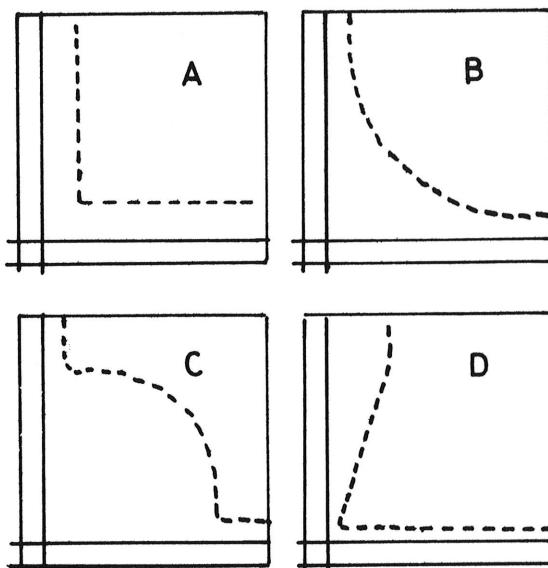
### MATERIALS AND METHODS

**Test fungus and growth conditions.**—Two wild-type strains of *Pyricularia oryzae*, Hoku-373 and Ken-6019, and PTL-resistant mutants, selected from these strains as reported previously (2), were used throughout these experiments. Wild-type strains were transferred twice a year to potato sucrose agar (PSA) slants, whereas PTL-resistant mutants were similarly transferred to PSA slants amended with 0.3 mM IBP. Tests were carried out at 27°C on PSA medium unless otherwise stated. Conidia of test strains used in the selection of mutants resistant to isoprothiolane and in evaluation of fungitoxicity were produced by the method previously reported (2); i.e., by placing a mycelial mat grown on a thin PSA plate onto another agar plate containing 0.2% yeast extract and 3% soluble starch followed by 4 days irradiation with near-ultraviolet light. Irradiations were performed with fluorescent black light with peak intensity at approximately 360 nm. The irradiation chamber

contained five 20 W fluorescent tubes (FL 20 BL), and the plates were placed about 40 cm below the tubes.

**Fungicides.**—Samples of IBP, edifenphos, and isoprothiolane were kindly supplied by Kumai Chemical Industry Co., Ltd., Nihon Tokushu Noyaku Seizo K. K. and Nihon Nohyaku Co., Ltd. respectively. Dihexyl *N*-methyl-*N*-phenylphosphoramidate (HPA) and dibutyl *N*-methyl-*N*-phenylphosphoramidate (BPA) were prepared and purified as reported previously (3).

**Selection of isoprothiolane-resistant mutants.**—Plates containing PSA amended with 100 µM (29 µg/ml) of isoprothiolane were inoculated uniformly with about 10<sup>6</sup>

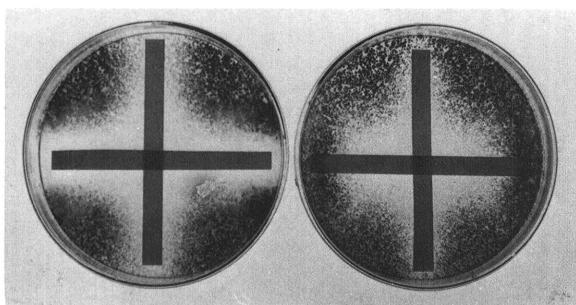


**Fig. 1-(A to D).** Four typical joint actions as demonstrated by the crossed-paper technique. Paper strips impregnated with test fungicides are shown by solid lines and the resultant growth-inhibitory zones are shown by dotted lines: A) independent action, B) additive action, C) synergism, and D) antagonism.

conidia per plate. Tolerant colonies were transferred to fungicide-free medium and single-spore isolates were made.

**Evaluation of fungitoxicity.**—Minimum growth-inhibitory concentrations were estimated from observations of mycelial growth on PSA plates containing 25, 50, 100, 200, 400, and 800  $\mu\text{M}$  of test fungicides 4 days after inoculation by streaking with conidial suspensions of test strains.

**Test method for joint action of fungicides.**—Joint action of IBP, isoprothiolane and BPA was estimated by a crossed paper technique (3, 4). A strip of filter-paper impregnated with a test fungicide was placed on PSA plates inoculated uniformly with conidia of a test strain. Another paper strip impregnated with the other fungicide



**Fig. 2.** Joint actions of isoprothiolane with dibutyl *N*-methyl-*N*-phenylphosphoramidate (BPA) and *S*-benzyl diisopropyl phosphorothiolate (IBP). Agar plates were inoculated uniformly with conidia of a wild-type strain of *Pyricularia oryzae*. Filter paper strips impregnated with the respective fungicides were placed on the plates to form filter paper cross. Additive effect in fungicidal action was presumed on the left plate between IBP (the horizontal strip) and isoprothiolane (the vertical strip) and synergism was shown on the right plate between BPA (the horizontal strip) and isoprothiolane (the vertical strip) by their patterns of growth inhibition.

was placed at a right angle to form a filter-paper cross. The growth-inhibitory zone around the paper strips was observed after 2 or 3 days. As shown diagrammatically in Fig. 1; a) two test fungicides having entirely different fungicidal action would not affect each other in the inhibitory zones; b) those having a similar mechanism of fungicidal action would form round corners at the crossing of the inhibitory zones of the two fungicides; c) a fungicide might produce a synergistic effect upon another fungicide and expand the inhibitory zone; and d) a fungicide might produce an antagonistic effect and cause a narrowing of the inhibitory zone.

## RESULTS

**Selection of isoprothiolane-resistant mutants.**—Growth of mycelia was not observed 4 days after inoculation of isoprothiolane-containing plates with conidia of wild-type strains. Six days after inoculation, however, the surface of the plates became covered with a thin translucent mycelial mat. In the growth of the original wild-type strain, a few small dense areas of mycelial growth were observed which was indicative of growth of mutant strains. These colonies were transferred to fungicide-free medium and subsequently single-spore cultures were isolated. All three clones, one from Hoku-373 and two from Ken-6019, thus obtained, proved to be isoprothiolane-resistant mutants.

**Fungicide sensitivity evaluation.**—The isoprothiolane-resistant mutants from Hoku-373 and Ken-6019 were resistant to PTL fungicides as well as to isoprothiolane, but were more sensitive to PA fungicides than were their parent wild-type strains. The PTL-resistant mutants obtained by selection from Hoku-373 and Ken-6019 were cross resistant to isoprothiolane (Table 1).

**Joint action of fungicides.**—With wild-type strains, the growth inhibitory zone around crossed paper strips impregnated, respectively, with isoprothiolane and BPA widened at the crossing point of the strips showing typical

**TABLE 1.** Sensitivity of wild-type strains and mutants of *Pyricularia oryzae* to phosphorothiolates, isoprothiolane, and phosphoramidates

Fungicides	Minimum inhibitory concentration ( $\mu\text{M}$ ) for		
	Wild-type strains <sup>a</sup>	PTL-R <sup>b</sup>	Isoprothiolane-R <sup>c</sup>
IBP	100	400	400
Edifenphos	25	100	100
Isoprothiolane	50	200	200
BPA	400	200	200
HPA	800	50	50

<sup>a</sup>Hoku-373 and Ken-6019 were used. Results were the same in either case.

<sup>b</sup>Mutants from Hoku-373 and Ken-6019 selected for resistance to organophosphorus thiolates.

<sup>c</sup>Mutants selected for resistance to isoprothiolane.

**TABLE 2.** Synergism of *S*-benzyl diisopropyl phosphorothiolate (IBP) and isoprothiolane with dibutyl *N*-methyl-*N*-phenylphosphoramidate (BPA) in fungicidal action on *Pyricularia oryzae* as estimated by crossed-paper technique

Fungicides	Synergism with BPA in fungicidal action on		
	Wild-type strains <sup>a</sup>	PTL-R	Isoprothiolane-R
IBP	yes	no	no
Isoprothiolane	yes	no	no

<sup>a</sup>Test strains Hoku-373 and Ken-6019 were used.

TABLE 3. Response of wild-type strains of *Pyricularia oryzae* and their mutants to fungicide treatment

Strains	Sensitivity <sup>a</sup> to	
	PTL and isoprothiolane	PA
Wild-type and revertants	S	R
Mutants not yet found	R	R
Mutants obtained	R	S

<sup>a</sup>Symbols: S = sensitive, R = resistant.

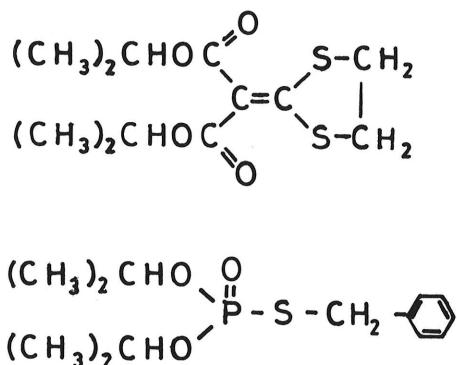


Fig. 3. Molecular structures of isoprothiolane (upper) and *S*-benzyl diisopropyl phosphorothiolate (IBP) (lower).

synergism in fungicidal action (Fig. 2, Table 2). The inhibitory zone of isoprothiolane and IBP showed rounding of corners which is common for additive effects of fungicides that have the same mode of action (Fig. 2). The pattern of synergism by BPA and isoprothiolane as well as by BPA and IBP was not observed with PTL-resistant mutants or isoprothiolane-resistant mutants (Table 2).

## DISCUSSION

A perfect correlation of cross resistance between PTL fungicides and isoprothiolane was shown in the present experiments, since all of the isoprothiolane-resistant mutants were cross resistant to PTL and PTL-resistant mutants were cross resistant to isoprothiolane. Furthermore, all mutants resistant to isoprothiolane and to PTL fungicides were more sensitive to PA fungicides than their parent wild-type strains. No difference in sensitivity to isoprothiolane, PTL, and PA fungicides was found among these mutants. Synergistic fungicidal action between isoprothiolane and PA fungicides, just as that between PTL and PA fungicides, was found in wild-type strains, but not in PTL-resistant and isoprothiolane-resistant mutants. These experimental facts strongly

suggest that mutants selected for PTL-resistance are identical with those selected for isoprothiolane-resistance. Although mutants resistant to both isoprothiolane and PA fungicides would probably be expected, they were not observed after selection with isoprothiolane from a large number of conidia of wild-type strains. All three mutants obtained in the present experiment were isoprothiolane-resistant and PA-sensitive. Similarly, we did not obtain mutants other than those which were PTL-resistant and PA-sensitive in the selection from wild-type strains with PTL fungicides (3), or mutants other than those which were PTL-sensitive and PA-resistant in the selection from PTL-resistant mutants with a PA fungicide (1) (Table 3). Thus, the negatively correlated cross resistance between PA fungicides and isoprothiolane or PTL fungicides seems to follow a consistent pattern. A single genetical factor, presumably one gene, seems to govern the resistance to PTL fungicides and isoprothiolane and also the sensitivity to PA fungicides. The evidence suggests a close relationship among isoprothiolane, PTL, and PA fungicides in their mode of action in *P. oryzae*. The chemical structure of isoprothiolane is seemingly different from that of PTL fungicides since it lacks a phosphorus atom and the sulfur atoms are in a thio-ether form rather than in a thio-ester form. However, the sulfur atoms in isoprothiolane are linked to a carbon joined by an  $\alpha$ ,  $\beta$ -unsaturated double bond next to carbonyl (Fig. 3). Thus, the thio-ether linkages of isoprothiolane probably resemble those of a thio-ester. This similarity in chemical structure may be the basis of similarity between isoprothiolane and PTL fungicides in their action on *P. oryzae*.

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