# いもち病菌に対する化学物質とホスホロチオール酸エステル殺菌剤の連合作用


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# Joint Action of Chemicals with Phosphorothiolate Fungicides on Rice Blast Fungus

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上杉康彦*・片桐政子*・野田　理**: いもち病菌に対する化学物質と
ホスホロチオール酸エステル殺菌剤の連合作用

**Abstract**

Organophosphorus compounds, *N*-methylcarbamates, amide compounds, alcohols, phenols and other compounds were tested for joint action with phosphorothiolate (PTL) fungicides by crossing the paper strips impregnated with the test chemicals on agar plate uniformly seeded with rice blast fungus.

Antagonism was found for phosphoramidothionate herbicide (amiprophos) and phosphorothiolothionate having carboxy ester structure (malathion and phenthoate). While synergism was found for some amide compounds, including propanil (3′, 4′-dichloropropinanilide) and its derivatives. There would be specificity of chemical structure in these amide compounds for synergism with PTL fungicide. The synergism was not observed when the PTL-resistant lines of the fungus was used as a test organism.

Mchanism of the synergism was discussed in connection with resistance of the fungus to PTL fungicides. (Received July 31, 1973)

## Introduction

During the course of the investigation of the resistance of rice blast fungus to organophosphorus fungicides, the cross-resistance to various chemicals was examined and resistant clone of the fungus to phosphorothiolate (PTL) fungicides was found to be specifically susceptible to phosphoramidates. These phosphoramidates showed low fungicidal activity to wild types of the fungus, but it has synergistic action with PTL fungicides to them[2,6].

On the other hand, an amide herbicide propanil, 3′, 4′-dichloropropionanilide, which is toxic to barnyard grass but not to rice plant, becomes toxic to rice plant when applied in combination with organophosphorus compounds or carbamates[3]. This means the synergism of propanil with these compounds on rice plant. Recently, another amide herbicide amiprophos, ethyl 2-nitro-*p*-tolyl *N*-isopropylphosphoramidothionate, was developed though it has not yet been put into practical use. It has nearly the same herbicidal spectrum as propanil, and has the chemical structure related to the above-described phosphoramidates.

These facts tempted the authors to test for joint action of these two amide herbicides with PTL fungicides to rice blast fungus, and the results was obtained that propanil showed synergism but amiprophos showed antagonism (Fig. 1). Thereafter, various chemicals are selected and tested for the joint action, from the standpoint of chemical structure-biological activity relationship.

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The results of the present investigation may help not only to elucidate the mechanism of their fungicidal action, resistance and joint action, but also to suggest the compatibility in combined use of pesticides with PTL fungicides.

## Materials and method

**PTL fungicides** *S*-Benzyl diisopropyl phosphorothiolate (Kitazin P) was used throughout the present experiment. Other PTL (including phosphonothiolate) fungicides, i. e. edifenphos (ethyl *S*, *S*-diphenyl phosphorodithiolate, Hinosan), *S*-benzyl ethylphenylphosphonothiolate (Inezin), and *S*-benzyl butyl *S*-ethyl phosphorodithiolate (Conen), were also tested for the joint action with the representative chemicals. These PTL fungicides were kindly supplied by respective manufacturers.

**Test chemicals** Organophosphorus insecticides, carbamate insecticides, amide herbicides and fungicides (mainly for rice blast control) were kindly supplied by respective manufacturers. Other methylcarbamates were synthesized and kindly supplied by Dr. Kuwatsuka of Nagoya University. Some of them have considerable protective effect against rice blast[1]. Propanil derivatives and fatty acid *N*, *N*-ethylenediamides were prepared and provided through the courtesy of Hodogaya Chemical Co., Ltd.. WARF antiresistant (*N*, *N*-dibutyl-*p*-chlorobenzenesulfonamide), the DDT-synergist to DDT-resistant insects, and other insecticide-synergists were synthesized and kindly supplied by Dr. Tomizawa of this institute. Alkyl chlorophenols were synthesized by the senior author. They are highly fungicidal to rice blast fungus especially at butyl to hexyl homologues[7]. Other test chemicals, e. g. alcohols and other amide compounds, are purchased as commercial reagents.

**Test fungi** An isolate of rice blast fungus, *Pyricularia oryzae*, coded as Hoku-373 was used throughout the experiment, and in some cases other isolate Ken-6019 was also used. The isolates and their PTL-resistant (PTL-R) clones were cultured and their spores were obtained by the method described in the previous paper[5]. The PTL-R clones were obtained by selection under the pressure of *S*-benzyl diethyl phosphorothiolate (Kitazin) in agar medium, and are cross-resistant to other PTL fungicides[5].

**Testing method for joint action of fungicdes** About $10^4$ spores of test fungus were seeded uniformly on potato sucrose agar plate in Petri dish of 9 cm diameter. A strip of filter paper of 7 mm width and 8 cm length was dipped in the 3 mM solution (acetone was generally used as solvent) of a test chemical, dried and placed on the agar plate. Another paper strip was also impregnated with a PTL fungicide and placed on the plate crossing at right angle to the paper strip of the test chemical. Growth-inhibition zone on the plate around the crossing paper strips was observed 2 days after the treatment and its pattern shows the mode of joint action. Some highly fungicidal chemicals, e. g. blasticidin S, kasugamycin, and most of the phenols tested, formed broad inhibition zone so that it became difficult to observe the pattern of the zone around the crossing point of the paper strips. In such cases, dosage impregnated in the paper strip was diminished in order to obtain a proper pattern of the inhibition zone. On the contrary, there were some cases that above-described dosage was insufficient for the observation of the joint action and increase of dosage gave rise to a clear pattern of joint action. When the dosage of 3 mM solution exhibit only slight joint action, the results were designated in small letter and listed on the Table 1 to 4, while clear joint actions at that dosage or lower were designated in capital letter. Patterns of inhibition zone around the crossing point of the paper strips indicating modes of joint action are as follows:

Independent action: When the two chemicals in the crossing paper strips have fungicidal actions based on different mechanisms and do not affect their activities each other, the inhibition zone around the crossing paper strips becomes a square corner.

Additive action: When the two chemicals has the same fungicidal mechanism, the inhibition zone is made by the additive action of the two chemicals and becomes a round corner.
Synergistic action: If there is synergism between the two chemicals, the inhibition zone widens at the crossing point of the two paper strips.
Antagonistic action: If there is antagonism, the zone is narrowed at the crossing point.

**Testing method for fungicidal activity** Fungicidal activity was tested by agar dilution method and expressed by minimum growth-inhibitory concentration (MIC)[5] evaluated 4 days after the test. Test concentrations in the potato sucrose agar medium are usually 0.1, 0.2, 0.4 and 0.8 mM.

## Results

The results are listed in Tables 1, 2 and 3. Since the practical joint action would not be so simple and it was observed qualitatively in the present experiment only by the appearance of the growth-inhibition zone on agar plate, the results on the tables show only an outline examined from one standpoint. Nevertheless, there would be a correlation between chemical structure and the joint action.

Table 1. Joint action of chemicals with PTL fungicides

| Chemical structure | Joint action[a] |
|---|---|
| (Amide herbicides) | |
| $(CH_3)_2CHNHP(S)(OC_2H_5)(OC_6H_3(NO_2)(2)(CH_3)(4))$ (amiprophos) | ANT |
| $C_2H_5CONHC_6H_3Cl_2(3,4)$ (propanil)[b] | syn |
| $CH_3OCONHC_6H_3Cl_2(3,4)$ (swep) | add-syn |
| (Carbamate insecticides)[c] | |
| $CH_3NHCOOC_6H_5OCH(CH_3)_2(o)$ (arprocarb, propoxur) | IND |
| $CH_3NHCOOC_{10}H_7(\alpha)$ (carbaryl) | add-syn |
| $CH_3NHCOOC_6H_2(CH_3)_2(3,5)(N(CH_2CH=CH_2)_2)(4)$ | SYN |
| (Organophosphorus insecticides) | |
| $(CH_3O)_2P(S)OC_6H_4NO_2(p)$ (parathion-methyl) | add-syn |
| $(C_2H_5O)_2P(S)OC_6H_4NO_2(p)$ (parathion) | add-syn |
| $(CH_3O)_2P(S)OC_6H_3(CH_3)(3)(NO_2)(4)$ (fenitrothion) | syn |
| $(CH_3O)_2P(S)OC_6H_3(CH_3)(3)(SCH_3)(4)$ (fenthion) | syn |
| $(CH_3O)_2P(O)SCH_2CH_2SC_2H_5+(CH_3O)_2P(S)OCH_2CH_2SC_2H_5$ (demeton-methyl) | IND |
| $(CH_3O)_2P(O)SCH(CH_3)CH_2S(O)C_2H_5$ | IND |
| $(C_2H_5O)_2P(S)SCH_2CH_2SC_2H_5$ (disulfoton) | add |
| $(CH_3O)_2P(O)CH(OH)CCl_3$ (trichlorfon) | IND |
| $(CH_3O)_2P(S)SCH(COOC_2H_5)CH_2COOC_2H_5$ (malathion) | ant |
| $(CH_3O)_2P(S)SCH(C_6H_5)COOC_2H_5$ (phenthoate) | ANT |
| (Fungicides for rice blast control) | |
| $(CH_3COOHgC_6H_5$ (PMA) | IND |
| $\ulcorner C_6Cl_4-C(O)OCH_2 \urcorner$ (tetrachlorophthalide) | add-syn |
| $\ulcorner C_6H_4-C(OCH_2CH=CH_2)=NSO_2 \urcorner$ (propenazole) | IND-add |
| blasticidin S | ADD |
| kasugamycin | ADD |

Table 1. (continued)-1

| Chemical structure | Joint action[a)] |
|---|---|
| (Insecticide synergists and related compounds) | |
| $-OCH_2OC=C-CH=C(n\text{-}C_3H_7)-C(CH_2(OCH_2CH_2)_3C_2H_5)=CH$ (piperonyl butoxide) | add |
| $(p)ClC_6H_4SO_2N(n\text{-}C_4H_9)_2$ (WARF antiresistant) | add |
| $(p)ClC_6H_4SO_2N(CH_3)C_6H_4Cl(p)$ | ADD |
| $(p)CH_3C_6H_4SO_2NH\text{-}n\text{-}C_4H_9$ | syn |
| (Alcohols and phenols) | |
| $CH_3(CH_2)_nOH$ $n=5, 7, 9, 11, 13$ | IND |
| $HOC_6H_3(Cl)(4)((CH_2)_nCH_3)(2)$ $n=4, 5, 7$ | ADD |
| " $n=9$ | add-IND |
| $C_6Cl_5OH$ (PCP) | ADD |
| (Oher carboxamides) | |
| $CH_3CONH_2$ | IND |
| $CH_3CONHCH_3$ | IND |
| $C_6H_5CONH_2$ | IND |
| $CH_3CONHC_6H_5$ | IND-syn |
| $C_6H_5CONC_6H_5$ | syn |
| $-(CH_2)_n-C(O)NH$ $n=3, 4, 5$ | add-syn |
| $CH_3(CH_2)_nCONHCH_2CH_2NHCO(CH_2)_nCH_3$ $n=0, 1, 2, 3, 4$ | IND |
| " $n=5$ | IND-syn |
| " $n=6, 8, 10, 12$ | IND |
| $NH_2CH_2CONHCH_2COOH$ | IND |
| $NH_2CH_2CONHCH_2CONHCH_2COOH$ | IND |
| $NH_2CH_2CONHCH(COOH)CH_2CH(CH_3)_2$ | IND |
| $(CH_3)_2CHCH_2CH(NH_2)CONHCH_2COOH$ | IND |
| $NH_2CH_2CONHCH(COOH)CH_2C_6H_4OH(p)$ | IND |
| $(CH_3)_2CHCH_2CH(NH_2)CONHC_{10}H_7(\beta)$ | syn |

a) IND: independent, ADD: additive, add: slightly additive, SYN: synergistic, syn: slightly synergistic, ANT: antagonistic, ant: slightly antagonistic.
b) The joint actions of the related compounds are listed on Table 2.
c) The joint actions of other *N*-methylcarbamates are listed on Table 3.

**Amide herbicides** The results with amiprophos and propanil are in marked contrast as shown in Fig. 1 and Table. 1. The antagonism of amiprophos to PTL fungicides forms also a pronounced contrast with the synergism of phosphoramidates, structurally related to amiprophos (Table 4).

Some of chloro-substituted anilides of fatty acids, i. e. derivatives of propanil, showed the synergism even more remarkably than propanil. Both the position of chloro-substitution and the length of fatty acid moiety are important for the joint action, and the synergistic action was remarkably shown when fatty acid was butyric or hexanoic acid and the chlorines were substituted on both the 3- and 4-positions of the benzene ring (Table 2).

Another herbicide swep, an amide of monoester of carbonic acid, i. e. a carbamate, was also somewhat synergistic to the PTL fungicide.

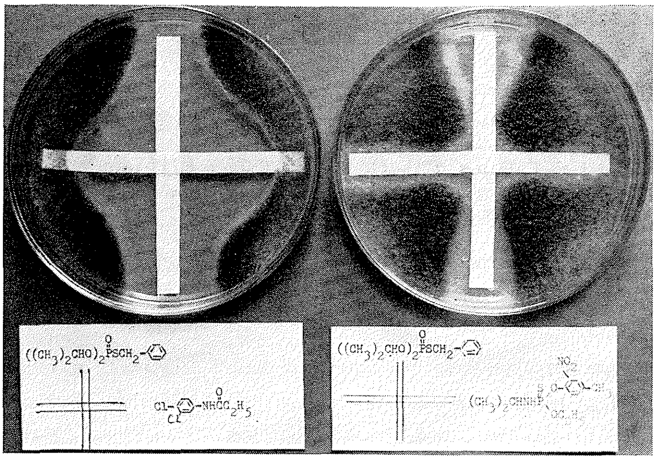


Fig. 1. Synergism of propanil (the horizontal paper strip on the left plate) and antagonism of amiprophos (the horizontal paper strip on the right plate) to *S*-benzyl diisopropyl phosphorothiolate (the vertical paper strips).

Table 2. Joint action with PTL fungicides of chloro-substituted anilides of fatty acids. RCONH—⟨benzene⟩$Cl_n$

| R | Substitution ($Cl_n$) | | | | | |
|---|---|---|---|---|---|---|
| | none | *o*-Cl | *m*-Cl | *p*-Cl | 3, 4-$Cl_2$ | 2, 5-$Cl_2$ |
| H | IND | — | — | — | — | — |
| $CH_3$— | — | — | — | — | IND-syn | — |
| $C_2H_5$— | IND | IND | IND | IND | syn | IND |
| $CH_3(CH_2)_2$— | — | — | IND | syn | SYN | — |
| $CH_3(CH_2)_4$— | — | — | — | — | SYN | — |
| $CH_3(CH_2)_7$— | — | — | — | — | IND-add | — |

***N*-methylcarbamates** Some carbamate insecticides showed synergism with the PTL fungicide. Substituted phenyl methylcarbamates having comparatively simple substituents were, therefore, examined for the joint action (Table 3). Chloro- and nitro-substitution exhibited the additive or synergistic action, while methyl- and methoxy-substitution did not exhibit the joint action. Methyl-substituent might, however, promote the joint action of chloro- or nitro-substituted phenyl carbamates. Such effects of substituents on the joint action was seemingly more remarkable on the 3-, 4- and 5-positions than on the 2-position of the benzene ring. 3, 4, 5-Trisubstituted phenyl methylcarbamates sometimes showed strong synergism. Though effect of amino-substituent has not been tested, 3, 5-dimethyl-4-(diallylamino)-substituted one is also markedly synergistic.

**Organophosphorus insecticides** The compounds tested could be divided into 3 groups as suggested from the difference of their chemical structures. Substituted phenyl phosphorothionates including parathion, parathion-methyl, fenitrothion and fenthion were more or less synergistic. The second group is trialkyl derivatives, i. e. trialkyl esters (demeton-methyl, disulfoton, etc.) and dialkyl alkylphosphonate (trichlorfon). These compounds generally did not show joint action, though disulfoton showed slight additive action. The third group showed the antagonistic action and to this group belong structurally related two compounds, malathion and phenthoate, which have *S*-ethoxycarbonylmethyl *O*, *O*-dimethyl phosphorothiolothionate structure.

Table 3. Joint action with PTL fungicides of substituted phenyl *N*-methylcarbamate. $CH_3NHCOO$—  $R_n$

| Substitution ($R_n$) | | | | Joint action with PTL fungicides |
|---|---|---|---|---|
| 2 | 3 | 4 | 5 | |
| — | — | — | — | IND |
| $CH_3$ | — | — | — | IND |
| — | $CH_3$ | — | — | IND |
| — | — | $CH_3$ | — | IND |
| $CH_3O$ | — | — | — | IND |
| — | $CH_3O$ | — | — | IND |
| — | — | $CH_3O$ | — | IND |
| Cl | — | — | — | add |
| — | Cl | — | — | add |
| $C_6H_5$ | — | — | — | add |
| — | — | $C_6H_5$ | — | ADD |
| — | — | $NO_2$ | — | syn |
| $CH_3$ | — | $CH_3$ | — | add |
| $CH_3$ | — | — | $CH_3$ | IND |
| — | $CH_3$ | — | $CH_3$ | add |
| — | $CH_3$ | Cl | — | add |
| — | $CH_3$ | $NO_2$ | — | syn |
| — | Cl | Cl | — | syn |
| — | $CH_3$ | Cl | $CH_3$ | SYN |

**Fungicides** Organomercury fungicide PMA did not show any joint action, while two antibiotics, blasticidin S and kasugamycin, showed additive action with the PTL fungicide. Tetrachlorophthalide, which has cyclic ester structure, has some tendency to synergism.

**Insecticide synergists and related compounds** A pyrethrin synergist, piperonyl butoxide, has some additive action to the PTL fungicide. WARF antiresistant also showed only slight additive action, while its structurally related compound *N*-butyl-*p*-toluenesulfonamide has synergistic action to the PTL fungicide.

**Alcohols and phenols** During the course of the study it was suggested that a proper lipophilicity may be necessary for a compound to be synergistic to PTL fungicides. Homologous series of compounds were, therefore, examined for joint action. Series of alcohols and 2-alkyl-4-chlorophenols did not show any synergism. Therefore, it is clear that a proper lipophilicity is not the sufficient condition, though it might be a supplementary factor for synergism with PTL fungicides. It was also noteworthy that phenols showed generally the typical pattern of additive action with the PTL fungicide.

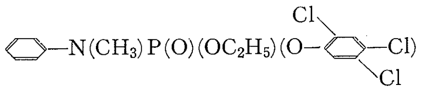
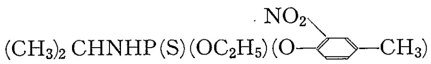
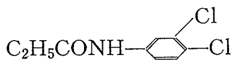
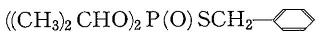
**Other carboxamides** Since amide herbicides showed characteristic joint action and some of carbamates also showed synergism with PTL fungicides, further examination was carried out with various carboxamides, and some of them exhibited synergism with the PTL fungicide. Though many hydrophilic peptides, acetamide and benzamide did not show any joint action, two rather lipophilic amides, benzanilide and *N*-$\beta$-naphthylamide of alanine, showed synergism with the PTL fungicide. The results with ethylenediamides of fatty acids indicate, however, that lipophilicity and amide structure were not the sufficient factors for synergism, because none of the members exhibited clear synergism.

These results suggest that some structural specificity must be needed for amide compounds to be synergist with PTL.

**The test with other PTL fungicides** 3, 4-Dichloroanilides of fatty acids and amiprophos were tested for joint action also with three PTL fungicides other than *S*-benzyl diisopropyl phosphorothiolate, and the similar results were obtained. These PTL fungicides tested have, of course, additve action with each other.

**Cross-resistance with PTL fungicides and the joint action on PTL resistant clones of the fungi** The representative synergists or an antagonist as listed in Table 4 were examined for the joint action and fungicidal activity on wild type of the fungi as well as on their PTL-R clones. The results on the two test isolates, Hoku-373 and Ken-6019, were as shown in Table 4.

Table 4. Joint action with PTL fungicides and fungicidal activity of some representative amide compounds on wild type of rice blast fungus and its PTL-resistant clone.

| Test chemicals | Joint action with PTL | | Fungicidal activity MIC (mM) | |
|---|---|---|---|---|
| | wild type | PTL-R | wild type | PTL-R |
|  | SYN | IND | 0.8 | ≦0.1 |
|  | ANT | IND | ≦0.1[a] | ≦0.1[a] |
|  | syn | IND | 0.4 | 0.4 |
|  | SYN | IND | 0.2 | 0.2 |
|  | ADD | add | 0.1 | 0.4 |

a) *> 0.8 after 7 days.

Synergism and antagonism were observed only on wild types of the fungus and not on their PTL-R clones. Negative correlation in cross-resistance was observed between PTL fungicides and phosphoramidates as previously reported[2,6], while no correlation in cross-resistance was observed between PTL fungicides and the other synergists or the antagonist.

## Discussion

Synergism and negative correlation in cross-resistance between phosphoramidates and PTL fungicides in their fungicidal action on rice blast fungus have been reported and these phenomena were anticipated to be a clue to elucidate the mechanism of resistance and the fungicidal mechanism of PTL fungicides[2,6]. The present investigation adds another datum that some amide compounds are also synergistic to PTL fungicides on wild type of the fungus though they were different from the action of phosphoramidates in having no correlation in cross-resistance with PTL fungicides.

Synergism in pesticidal action have often been proved to be based on the inhibition of detoxification of pesticide by synergist. If this type of synergism occurs between the amide compounds and PTL fungicides, one of the synergistic pair would be detoxified by the enzyme system which is susceptible to inhibition by the other member of the pair in the cells of wild type of rice blast fungus, but this detoxification and/or inhibition would not take place in the PTL-R clones of the fungus. The metabolism of PTL fungicides by a wild type of the fungus and its PTL-R clone have been studied and no difference in metabolism was found between the two lines of the fungus[4,8,9]. This result suggests that susceptibility of the fungus to PTL fungicide did not depend on detoxification or activation. There are, therefore, one possibility that the pesticide that is to be synergized is not the PTL fungicide but the amide compound, and the synergist is the PTL fungicide. Structure-specificity of amide compounds for the synergism might be an evidence that the amide compounds exhibit synergism with PTL fungicides only when they can be substrates of enzyme system which detoxifies them. From this point of view, examinations of the metabolism of the amide compounds by the wild type and the PTL-R fungi and the inhibition of the metabolism by PTL fungicides remain to be solved.

Absence of negative correlation in cross-resistance between PTL fungicides and amide compounds other than phosphoramidates suggests a difference in fungicidal mechanism or a very delicate difference in metabolism (detoxification and/or activation) between phosphoramidates and other amide compounds.

Antagonism to PTL fungicides was found with a phosphoramidothionate and two (*S*-alkoxycarbonylalkyl) dialkyl phosphorothiolothionates. These antagonists to PTL fungicides have chemical structure related to the synergists, i. e. phosphoramidates and substituted phenyl dialkyl phosphorothionates respectively. These facts suggest some relation between antagonist and synergist.

Anyway the facts revealed in the present study will serve for elucidation of mode of action of PTL fungicides and resistance mechanism to them, and may give a useful information for the practical protection of rice from the blast fungus.

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**和文摘要**

# いもち病菌に対する化学物質とホスホロチオール酸エステル殺菌剤の連合作用

上杉康彦・片桐政子・野田　理

薬理上および実用上の資料を得る目的で，チオールエステル型有機りん殺菌剤と各種薬剤の間のいもち病菌に対する殺菌連合作用を調べた。

酸アミド型除草剤，有機りん殺虫剤，*N*-メチルカルバミン酸エステル，いもち病防除薬剤，その他の酸アミド，アルコール類およびフェノール類などについてろ紙交さ法によって試験した結果，プロパニル (3′,4′-ジクロルプロピオンアニリド) およびその誘導体などアミド系化合物に有機りん殺菌剤と協力作用を示すものがあり，チオノ型置換フェニルエステル構造の有機りん殺虫剤にも協力作用の傾向が見られた。これら化合物が協力作用を示すには化学構造特異性が必要であるように思われる。一方，ホスホロアミドチオン酸エステルやカルボン酸エステル構造を有するチオールチオノ型有機りん殺虫剤など，協力剤に近似した化学構造の薬剤に有機りん殺菌剤との拮抗作用が見られた。

これらの連合作用は，有機りん殺菌剤耐性のいもち病菌を供試した場合は観察できなかった。また，本試験で見出された協力剤および拮抗剤と有機りん殺菌剤との間には交差耐性の正負いずれの相関も見られなかった。

いもち病菌の有機りん剤耐性に関する過去の試験成績と本実験結果をあわせて考察し，有機りん殺菌剤とアミド系化合物などとの間の協力作用は，後者に対する前者の協力作用である可能性を指摘した。