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Phytoalexin Induction by Some Agricutural Fungicides and Phytotoxic Metabolites of Pathogenic Fungi*

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Introduction

Phytoalexins, antifungal substances produced by infected host plants are known to be related to the defence mechanism of plants against diseases. These substances are also produced in response to the stimulation by non-living materials, such as spore-free germination fluids (CRUICKSHANK and PERRIN, 1963), metabolic inhibitors (CONDON and KUĆ, 1963, PERRIN and BOTTOMLEY, 1962), metalic ions (CRUICKSHANK and PERRIN, 1963), ethylene (CHALUTZ et al., 1969, CHALUTZ and STAHMANN, 1969), and antibiotics (SCHWOCHAU and HADWIGGER, 1968).

Some organo-mercuric (UEHARA, 1963) and other synthetic organic fungicides (OKU and NAKANISHI, 1964, REILLY and KLARMAN, 1972) were reported to be the effective phytoalexin inducers and these facts seem to suggest the possibility that fungicides exert protective functions not only by their direct antifungal activities, but also through their effects on the physiological processes in host plants.

This paper deals with the pisatin-inducing abilities of some representative commercial fungicides and fungal phytotoxins and aims to envisage the possibility to develop new types of plant disease control agents.

Materials and Methods

Ten grams of pea pods harvested from the field-grown pea plants (Alaska pea) just before the perfect maturation were placed in a moistend petri-dish of 15 cm-diameter and 4 ml of test compound solutions of appropriate concentration were placed on the endocarp of the pods. The solution of test compounds were recovered after 24 hr incubation at 22°C, and the pisatin induced into the solution was extracted twice with petroleum ether. The extract was concentrated *in vacuo* to dryness and the residue was dissolved in 4 ml of ethanol.

The UV absorption spectra of the ethanol solution were determined and the amount of pisatin induced by each compound was calculated by the method of CRUICKSHANK and PERRIN (1961) from the optical density at 310 nm.

Results

The experimental results obtained were summarized in Table 1. Organic fungicides, triazin, dichlone, and triphenyltin compounds induced respectively considerable amount of pisatin. Phenylmercury acetate was a effective inducer of pisatin as UEHARA (1963)

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Table 1. Pisatin induction by some agricultural fungicides and phytotoxic metabolites of pathogenic fungi.

Compound	Pisatin induced (μg/ml) at concentration of compounds (μg/ml)					
	1	3	10	30	100	300
Triazine	—	—	—	20		
Dichlone	—	—	—	3.5	6	
Phenylmercury acetate (PMA)	7	11	23	18	12	
UV-degradation product of PMA	4	11	—	—	—	
Kitazin P	—	—	—	—	5	
Tetramethylthiuram disulfide	—	—	—	—	—	
Thiophanate	—	—	—	—	—	
Triphenyltin acetate	—	10	6	—	—	
Triphenyltin hydroxide	—	7	5	7	2	
Captan	—	—	—	—	5.5	
Terrazole	—	—	—	—	—	
3-Hydroxy-5-methyl isoxazole (Tachigaren)	—	—	—	7	17	10
Blasticidin S	—	—	—	—	—	
Cycloheximide	27	34	9	—	—	
Ascochitine	—	—	12	13	24	
Ophiobolin	—	7	7	11	2	

— : Not detected.

pointed out. The UV degradation product of phenylmercury acetate also produced pisatin. Ascochitine and ophiobolin, metabolites of *Ascochyta fabae* and *Cochliobolus miyabeanus* respectively, were also pisatin inducers.

Discussion

Phenylmercury acetate is a effective protectant of rice plant against the blast disease. In view of the instability of phenylmercury acetate to UV-irradiation the antifungal activity of this compound could hardly be the only mechanism operating in blast disease control. For example, irradiation of phenylmercury acetate at the dose of 4.2×10^{6} erg. mm^{-2} of UV ray caused an decrease of its antifungal activity to 1/70 of the non-irradiated control (OKU, unpublished data). In the present experiment we found that both phenylmercury acetate and the UV-irradiated degradation product were potent inducers of phytoalexin and these facts may be of help in understanding how this UV-unstable phenylmercury acetate acts as an excellent protectant of rice blast disease even in the sunny fields. Unfortunately, our experiments were done with pea plant and not rice, but NASUDA (1973) demonstrated an antifungal metabolite in the exudate from phenylmercury acetate-splayed rice leaves.

Some other agricultural fungicides also induced a phytoalexin, pisatin in the endocarp of pea pod and these results supply additional supports to the concept that the physiological effects of fungicides on host plant may, in addition to the direct fungicidal actions, play some roles in plant disease control.

Phytotoxic metabolites of plant pathogenic fungi, ascochitine and ophiobolin, also induced pisatin. These toxins are the metabolites of *Ascochyta fabae* (OKU and NAKANISHI, 1963) and *Cochliobolus miyabeanus* (OKU, 1967) respectively and the chemical structures have already been determined. These are low molecular weight substances unlike the other phytoalexin inducer of fungal origin such as Monilicolin A reported by CRUICKSHANK and PERRIN (1968). Recently, BRIDGE and KLARMAN (1973) reported that the soybean phytoalexin, hydroxyphaseollin, was induced by ultraviolet irradiation when plants were maintained in darkness, and the irradiated plants became less susceptible to the soybean pathogen, *Phytophthora megasperma* var. *sojae*.

It would be useful to search compounds which accerelate the phytoalexin induction for the development of harmless agricultural chemicals, especially of the nature-bound fungicides, to control plant diseases.

Summary

The endocarp of the fresh pea pod incubated with solution or suspension of agricultural fungicides, phytotoxic metabolites of plant pathogenic fungi formed the pea phytoalexin, pisatin. Among the compounds tested, cycloheximide, triazine, dichlone, phenylmercury acetate, UV degradation product of phenylmercury acetate, triphenyltin fungicides, and 3-hydroxy-5-methylisoxazole induced pisatin. Ophiobolin, a toxin from *Cochliobolus miyabeanus*, and ascochitine, a toxic metabolite from *Ascochyta fabae*, also induced pisatin. The possibilities of the development of harmless plant disease control agents was discussed in relation to the induced synthesis of phytoalexins.

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農業用殺菌剤，植物病原菌の有毒代謝産物による ファイトアレキシンの生産

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エンドウの莢を種々の農業用殺菌剤，植物病原菌の毒素で処理して，エンドウのファイトアレキシンであるピサチンの生産される状態をしらべた．実験に供した種々の殺菌剤のなかで，フェニール酢酸水銀およびその紫外線による分解産物，トリアジン，ヂクロン，有機錫殺菌剤，タチガレン原体の 3-hydroxy-5-methylisoxazole，シクロヘキシミド等がピサチンを生産した．イネゴマハガレ病菌の代謝毒素，オフィオボリン，ソラマメ褐斑病菌の有毒代謝産物，アスコキチンもピサチンを生産した．このようなことから，直接殺菌性を持たないで，植物にファイトアレキシンを生産させるような性質の，無害な病害防除薬剤開発の可能性を論じた．