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On the mode of action of the organophosphorus fungicide Hinosan

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Since the introduction of triamiphos against powdery mildew fungi (van den Bos et al., 1960) a number of other organophosphorus fungicides have been developed and used in practice. Among them are compounds especially active against diseases on rice, e.g. Kitazin-P (*O,O*-diisopropyl *S*-benzylphosphorothiolate) and Hinosan (*O*-ethyl *S*, *S*- diphenylphosphorodithiolate), while others are particularly active against diseases caused by powdery mildew fungi, e.g. pyrazophos (Curamil, Hoe 2873, *O,O*-diethyl *O*-(6-ethoxycarbonyl-5-methylpyrazolo [1,5-a] pyrimid-2-yl) phosphorothionate) (Mariouw Smit, 1969; Uesugi, 1970). The mode of action of Kitazin-P has been studied by Maeda et al. (1970). In our studies on organophosphorus fungicides we compared the effects of Hinosan and pyrazophos with those of Kitazin (De Waard, 1971). Results of experiments with Hinosan are presented in this communication.

Methods used were those of Maeda et al. (1970) with slight modifications. Mycelium of *Piricularia oryzae* was incubated with D-glucosamine-1-^{14}C hydrochloride in a 2% glucose 0.5% yeast extract medium, and centrifuged and washed after 2 hours of incorporation. This resulted in a supernatant and a mycelial pellet, which was extracted overnight in 5% trichloroacetid acid (TCA) and then centrifuged again; this provided a TCA-extract and a mycelial residue. The three fractions thus obtained were processed further according to Maeda et al. (1970). Supernatant, TCA-extract and mycelial residue were assayed for radioactivity. The fractions contained residual free glucosamine, acid-soluble intermediates of chitin synthesis, and cell wall material, respectively. Effects of Hinosan on ^{14}C-glucosamine incorporation were studied by adding 0.1 ml of a solution of Hinosan in acetone to 10 ml mycelial suspension, simultaneously with the addition of the radiochemical.

Results are presented in Fig. 1. Hinosan had a pronounced effect on the distribution of radioactivity in the different fractions. At a concentration of 10^{-4} *M* Hinosan an increase in the percentage of radioactivity present in the acid-soluble extract was noticed, while at higher concentrations of Hinosan the percentage of radioactivity in both TCA-extract and mycelial residue decreased. The decrease in these fractions paralleled an increase of the percentage of radioactivity in the incorporation medium. In Fig. 2, the dry weight increase of mycelial growth during the incorporation time is given. The accumulation of intermediates of chitin synthesis in the TCA-extract may be caused by an inhibition of chitin systhesis, as has been reported for the same fungus

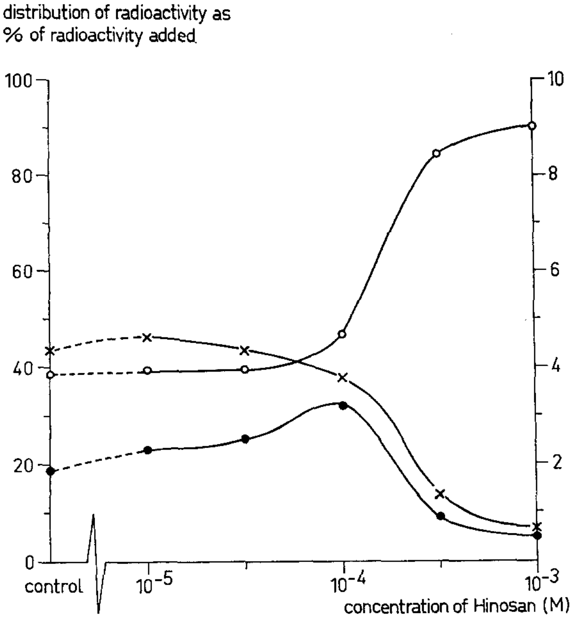

Fig. 1. Effect of Hinosan on incorporation of ^{14}C-glucosamine by mycelium of *Piricularia oryzae*. Supernatant of the incorporation medium (○ – ○), TCA-extract (● – ●), mycelial residue (× – ×). Percentages of radioactivity incorporated in TCA-extract are expressed on a 10 × enlarged scale.

Fig. 1. Effect van Hinosan op de incorporatie van ^{14}C-glucosamine door mycelium van Piricularia oryzae. Supernatant van het incorporatiemedium (○—○), TCA-extract (●—●), myceliumresidu (× – ×). Percentages radioactiviteit, geïncorporeerd in het TCA-extract zijn weergegeven op een 10 × vergrote schaal.

by Maeda et al. (1970) for Kitazin-P and by Ohta (1970) for the antibiotic polyoxin D. However at the concentration of 10^{-4} *M* Hinosan, which caused maximal accumulation of ^{14}C-glucosamine in intermediates, glucosamine incorporation in cell wall material was hardly reduced, while mycelial growth was inhibited to about 32% of normal growth. This might mean that still other factors are involved in the inhibition of mycelial growth. The low radioactivity in the TCA-extract and in the mycelial

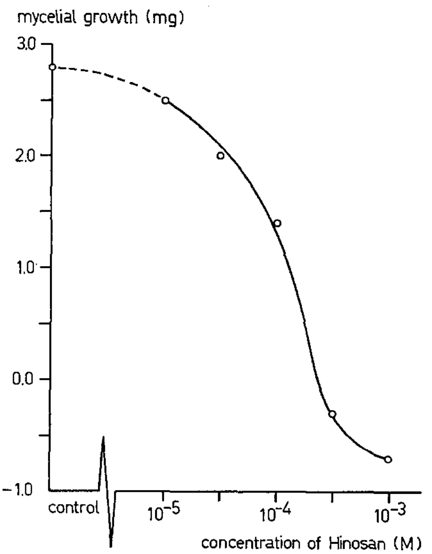

Fig. 2. Effect of Hinosan on mycelial growth of *Piricularia oryzae* during incorporation time. Initial dry weight 19.2 mg.

Fig. 2. Effect van Hinosan op de myceliumgroei van Piricularia oryzae gedurende de incorporatietijd. Aanvangsdrooggewicht 19.2 mg.

residue as well as the decrease in mycelial weight of samples incubated with 10^{-3} *M* Hinosan might indicate that also membrane permeability is influenced by the fungicide. A leakage of the cell membrane might prevent uptake of glucosamine and accumulation of intermediates of chitin synthesis.

This leakage can be related directly with a complete inhibition of mycelial growth, an assumption which is consistent with results obtained. Therefore, the fungicidal action of Hinosan can be considered to be based on an inhibition of chitin synthesis or on interference with cell membrane permeability. In view of the results obtained with Kitazin-P by Maeda et al. (1970) it is probable that the interference with cell wall permeability is primarily responsible for the fungitoxicity of the compound and that the inhibition of chitin synthesis has to be regarded as an indirect effect. This can be established by studying the effect of Hinosan on chitin synthesis in vitro.

Samenvatting

Over het werkingsmechanisme van de fungicide organische fosforverbinding Hinosan

In onderzoek naar werkingsmechanismen van fungicide organische fosforverbindingen werd aangetoond dat Hinosan bij *Piricularia oryzae* tussenprodukten van de chitinesynthese kan doen ophopen. Dit duidt op een beïnvloeding van de chitinesynthese. De verbinding heeft ook een effect op de permeabiliteit van de celwandmembraan. Waarschijnlijk is dit effect primair verantwoordelijk voor de fungitoxiciteit van Hinosan en moet de remming van de chitinesynthese als een indirect effect worden beschouwd.

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