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Effect of antimetabolites and fungicides on elongation of germination hyphae of powdery mildew in vitro

B. G. VAN 'T LAND and J. DEKKER

Laboratory of Phytopathology, Agricultural University, Wageningen

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Abstract

Elongation of germination hyphae of *Sphaerotheca fuliginea* on Czapek Dox agar with yeast extract continued for several weeks, resulting in the formation of hyphae with up to 30 cells. This permitted development of a method for measuring the effect on fungal growth in vitro of a number of fungicides and antimetabolites with known effect against powdery mildew diseases of plants.

Introduction

In recent years various compounds have shown systemic activity against powdery mildew diseases. As many of these are inactive against other fungi in vitro as well as in vivo, it is not known whether they exert a direct antifungal activity or act indirectly via the metabolism of the host plant. Procaine hydrochloride for example, a local anaesthetic without known antifungal activity, protects cucumber plants against powdery mildew after uptake by the roots (Dekker, 1961); L-methionine (Dekker, 1969) and kinetin (Dekker, 1963) prevent development of powdery mildew on leaf discs floating on solutions of these compounds.

In order to measure the direct effect of a chemical on powdery mildew fungi, spore germination tests may be used. This, however, provides only limited information, since it is known that the effect of a fungicide on spore germination may differ significantly from its effect on the growth of mycelium. Attempts have therefore been made to obtain development of powdery mildew hyphae on an agar medium, in order to permit assessment of the in vitro effect of systemic compounds upon these fungi.

Materials

The cucumber powdery mildew fungus, *Sphaerotheca fuliginea*, was maintained on cucumber plants, cv. 'Lange Gele Tros'. The chemicals 6-azauracil (AzU), 6-azauridine (AzUR), and kinetin were purchased from Nutritional Biochemicals Corporation, USA, 6-azauridine monophosphate (AzUMP) and D-methionine from California Corporation for Biochemical Research, USA, L-methionine from Sigma Chemical Company, USA, and procaine hydrochloride from The British Drug Houses Ltd., England. The fungicides were benomyl, technical pure, (methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate) from du Pont de Nemours & Co., Inc., USA; thiophanate, 50% W.P., (1,2-bis (3-ethoxycarbonyl-2-thioureido) benzene) and thiophanate-methyl, technical pure, (1,2-bis(3-methoxycarbonyl-2-thioureido)benzene) from Nippon Soda, Japan; ethirimol, 80%, (5-butyl-2-ethylamino-4-hydroxy-6-methyl-

pyrimidine) and dimethirimol, 10% W.P., (5-butyl-2-dimethylamino-4-hydroxy-6-methylpyrimidine) from Plant Protection Ltd., ICI, England; triamiphos, 95%, (phenyl-5-amino-1,2,4-thiazolyl-(1)-N,N^3-tetramethyldiamidophosphonate) from Philips-Duphar, The Netherlands; triforine, 100% (N,N'-bis(1-formamido-2,2,2-trichloroethyl)-piperazine) from C. H. Boehringer Sohn A. G., Germany; imugan, 95%, (1-(3,4-dichloroanilino)-2,2,2-trichloroethyl formamide) from Bayer A. G., Germany; triarimol, 4.65% (α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidine methanol) from Eli Lilly and Co., USA.

Elongation of germination hyphae in vitro

Cucumber seedlings were grown under sterile conditions by placing surface sterilized cucumber seeds on a Hoagland agar medium in 1 l erlenmeyers flasks containing 0.25% glucose. When cotyledons had developed they were excised and placed on agar medium in Petri dishes. From greenhouse-grown cucumber plants with sporulating powdery mildew, one or a few conidia were transferred with a sterile needle to each cotyledon. The plates were kept at room temperature and exposed to daylight or continuous illumination from fluorescent lamps with a total radiant energy of 1000 to 1500 lux. After selection of plates which appeared uncontaminated, cultures of powdery mildew were maintained by transfer of conidia to fresh sterile cotyledons every two or three weeks.

Conidia of *S. fuliginea* were dusted on a Czapek Dox agar medium (Oxoid CM 95) enriched with yeast 0.4% extract (Oxoid L 21) containing 0.8% agar (Oxoid L 13) with pH adjusted to 7.0. On this medium about 20-30% of the conidia germinated, a percentage which is much lower than De Waard (1971) obtained when conidia of the same fungus were dusted on cellulose membranes covering an agar nutrient medium. The latter method could not be used for our purpose, however, since elongation of germination hyphae on it soon ceased. On the above mentioned agar medium, growth of the germination hyphae continued for about 2-4 weeks, and the most rapidly developing germination hyphae (about one percent or less of the total population of conidia) produced 30 cells or even more per conidium. In each of four Petri dishes the five longest growing hyphae were selected and the number of cells in them counted. The average number of cells produced per conidium per day during the first two weeks was 2.3 and the average cell length 55 μm. Thereafter, growth was more or less irregular and hyphae became stunted. Measurement of growth was usually carried out during the first week of incubation, since after that period the mycelium became increasingly tangled, which hampered exact measurements. Haustoria were never observed in our cultures and no sporulation occurred.

Effect of chemical on powdery mildew in vitro

The chemicals to be investigated were incorporated in the agar medium and, after pouring the plates, conidia of cucumber powdery mildew were dusted on the surface of the agar and the plates incubated at 22°C. After five days the lengths of the five longest germination hyphae were measured and expressed as a percentage of the lengths of comparable hyphae on fungicide-free control media. The effect of the fungicides on the percentage germination of conidia was also determined, using agar

Table 1. Effect of fungicides and antimetabolites on germination of conidia and length of germination hyphae of *Sphaerotheca fuliginea*, expressed as percentages of controls; ED_{50}: approximate value.

	Germination									Germ tube elongation									
	10^{-2}	10^{-3}	10^{4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}M	ED_{50}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-5}M	ED_{50}
Procaine-HCl	33	75							4.10^{-3}	20	85	98							5.10^{-3}
D-methionine	74	71							$>10^{-2}$	30	60	95	100						2.10^{-3}
L-methionine	74	76							$>10^{-2}$	10	45	80	100	100					6.10^{-4}
AzU	65	61							$>10^{-2}$		5	15	60	95	100				2.10^{-5}
AzUR	100	88							$>10^{-2}$	5	15	75	90	100					2.10^{-4}
AzUMP	100	100							$>10^{-2}$			95	95	100	100				$>10^{-4}$
Kinetin		14	44	54	81				2.10^{-5}			10	10	50	85	95			10^{-6}
Triamiphos			39+	88+	94+				5.10^{-5}		5	35	90	95	95				5.10^{-5}
Triforine	27	82	75						2.10^{-3}			20	50	80	95	100			10^{-5}
Imugan		23	59	85	100				2.10^{-4}			10	25	40	70	90			6.10^{-7}
Thiophanate		3	0	50	80				10^{-5}			10	10	45	90	95			7.10^{-7}
Thiophanate-methyl		0	29	51	91				2.10^{-5}			10	10	45	85	95			7.10^{-7}
Benomyl		5	35	76	100				4.10^{-5}			10	10	15	55	95	100	100	2.10^{-7}
Triarimol		42	49	61	97				10^{-4}			5	30	35	40	45	85	100	10^{-7}–10^{-8}
Ethirimol				0	0	6	33	82	5.10^{-9}					10	10	20	80	100	5.10^{-9}
Dimethirimol				0	26	56	63	100	10^{-7}			10	10	25	75	100			3.10^{-7}

+ Data from de Waard, 1971.

Tabel 1. Effect van fungiciden en antimetabolieten op de kieming van conidiën en de groei van kiemhyfen bij Sphaerotheca fuliginea, in percentages van controle; ED_{50}: benaderde waarde.

covered with a cellpohane membrane, according to the method described by De Waard (1971). In these experiments the percentage germination of conidia on fungicide-free control media always exceeded 80%.

The data obtained are presented in Table 1, together with the estimated ED_{50} values.

Discussion

From the results it appears that the effect of fungicides on germ tube elongation may differ considerably from that on germination of conidia. In most cases germ tube growth was more sensitive to the fungicides than spore germination.

All commercial fungicides which have been tested inhibit germ tube elongation at low concentrations, the ED_{50} values ranging from 5×10^{-5} for triamiphos to 5×10^{-9} for ethirimol. In view of the concentrations needed for disease control, their effect in vivo might be due primarily to a direct effect on the fungus. The same might hold also for kinetin, although this compound is not known to inhibit in vitro growth of other fungi.

The concentrations of L-methionine and procaine hydrochloride, necessary to inhibit the elongation of the germ tubes in vitro, are rather high in relation to the concentrations which control powdery mildew on the plant. Unless, therefore, there is a marked accumulation of these substances in the epidermal cells, it seems difficult to attribute the effect against the disease to a direct effect on the fungus only; an indirect effect via the metabolism of the host plant might therefore be involved. D-methionine appears rather inactive against powdery mildew in vitro as well as in vivo.

Since it is known from other studies (Dekker, 1968) that not AzU, but its nucleotide AzUMP is the fungitoxic compound, it must be assumed that the powdery mildew fungus as well as the plant is able to convert AzU into AzUMP. The reduced effect of AzUR and the absence of direct fungicidal activity of AzUMP might be due to less penetration of these chemicals into the fungal cell, as has been demonstrated recently also for the fungus *Cladosporium cucumerinum* (Dekker, 1971).

Samenvatting

Effect van antimetabolieten en fungiciden op de groei van kiemhyfen van meeldauw in vitro

Na uitzaaien van conidiën van *Sphaerotheca fuliginea* op een agar medium, waaraan gistextract was toegevoegd, bleek groei van kiemhyfen zich bij een beperkt aantal conidiën gedurende enkele weken voort te zetten, waarbij tot 30 cellen werden gevormd. Dit verschafte een methode om de werking van fungiciden en andere verbindingen op meeldauwmycelium in vitro na te gaan. De resultaten werden vergeleken met die verkregen in sporekiemingstoetsen (Tabel 1).

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Address

Laboratorium voor Fytopathologie, Binnenhaven 9, Wageningen, the Netherlands.