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Toxicity of fenpropimorph to fenarimol-resistant isolates of Penicillium italicum

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Abstract

Fenarimol-resistant isolates of *Penicillium italicum* with cross-resistance to imidazole and triazole fungicides which inhibit ergosterol biosynthesis were tested for their sensitivity to fenpropimorph. Radial growth tests on PDA showed that the isolates (n = 6) lacked cross-resistance to fenpropimorph or displayed enhanced sensitivity to the fungicide (negatively correlated cross-resistance). Control of blue mold decay of oranges incited by the wild-type isolate could be achieved by dipping fruits in suspensions of fenpropimorph at a concentration of 100 mg ml^{-1}. Decay of oranges incited by the fenarimol-resistant isolates was controlled at the same or at a lower concentration (30 mg ml^{-1}), thus showing that the normal or increased sensitivity to fenpropimorph is also expressed under in vivo conditions.

Additional keywords: fungicide resistance, negatively correlated cross-resistance, Corbel, ergosterol-biosynthesis inhibitors.

Introduction

Ergosterol-biosynthesis inhibitors (EBI's) constitute an important group of systemic fungicides. Their antifungal mode of action is based on a site-specific inhibition of ergosterol biosynthesis. This implies the risk of development of EBI resistance in fungal populations. However, practical application of these fungicides has not led to failure in disease control. In contrast, in vitro selection of EBI-resistant mutants has often been reported (cf. Fuchs and De Waard, 1982; De Waard and Fuchs, 1982). Therefore, development of resistance under practical conditions remains a potential threat which deserves attention. In this context all means which may reduce the chance of development of resistance should be investigated. One of these is the use of chemicals to which EBI-resistant isolates have a normal or even increased sensitivity. The latter phenomenon has recently been reported for fenarimol-resistant isolates of *Penicillium italicum* with respect to fenpropimorph (De Waard er al., 1982). The present study describes this finding in more detail.

Materials and methods

Fungal strains. The *P. italicum* isolates used were W5 (wild-type) and A10-9, B10-4, C10-8, D100-4, E300-3 and E300-5 (fenarimol-resistant). They have been characterized previously (De Waard et al., 1982).

Chemicals. Fenarimol (technically pure) and Rubigan (12% EC fenarimol) were generously supplied by Lilly Research Centre Limited (Surrey, England) and Eli Lilly Nederland (Utrecht, the Netherlands); fenpropimorph (technically pure) and Corbel (75% EC fenpropimorph) by Dr R. Maag AG (Dielsdorf, Switzerland). Stock solutions of fenarimol were made in methanol and of fenpropimorph in water after mixing the fungicide with three parts (w/w) of lactic acid.

Toxicity assays. Toxicity of fenarimol and fenpropimorph to radial growth was determined on PDA amended with the fungicides at various concentrations. The EC_{50} values derived from dosage-response curves were used to characterize the sensitivity of each of the isolates. In addition, EC_{100} values, which represent fungicide concentrations at which radial growth was fully inhibited, are given. The efficacy of Corbel against *Penicillium* decay of oranges was tested by curative dip treatments of the fruits, 24 h after inoculation (De Waard et al., 1982).

Results

In vitro experiments. EC_{50} and EC_{100} values indicate that almost all fenarimol-resistant isolates of *P. italicum* tested, had an increased sensitivity to fenpropimorph as compared with the wild-type isolate (Table 1). Isolate C10-18 which displayed the lowest degree of resistance to fenarimol, had the same sensitivity to fenpropimorph as the wild-type isolate. In none of the isolates tested positively correlated cross-resistance to fenarimol and fenpropimorph was observed.

Table 1. Toxicity of fenarimol and fenpropimorph to radial growth of wild-type W5 and fenarimol-resistant isolates of *Penicillium italicum.*

	Isolate						
	W5	A10-9	B10-4	C10-18	D100-4	E300-3	E300-5
Fenarimol							
EC_{50}	0.30[1]	7.9	5.8	4.6	5.7	15.0	10.0
EC_{100}	10	300	100	30	300	>300	>300
Fenpropimorph							
EC_{50}	0.074	0.030	0.022	0.072	0.042	0.038	0.038
EC_{100}	1.0	0.3	0.3	1.0	0.3	0.3	0.3

[1] Fungicide concentration in $\mu g\ ml^{-1}$.

Tabel 1. Toxiciteit van fenarimol en fenpropimorf voor de radiale groei van het wild-type W5 en fenarimol-resistente isolaten van Penicillium italicum.

In vivo experiments. Three isolates (A10-9, C10-18 and E300-3) with different levels of resistance to fenarimol were selected for the in vivo tests. Oranges were inoculated with these isolates and after 1 day dipped in suspensions of Corbel (a.i. fenpropimorph) at different concentrations. Results of the experiments (Table 2) indicate that the ef-

Table 2. Effect of curative dip treatments of oranges with formulated fenpropimorph (Corbel) against decay caused by wild-type W5 and fenarimol-resistant isolates of *Penicillium italicum.*

Exp.[1]	Isolate	Fenpropimorph (μg a.i. ml^{-1})				
		0	10	30	100	300
1	W5	29[2] (48)	19 (44)	7 (25)	0 (10)	0 (4)
	A10-9	28 (51)	3[3] (12)[3]	0[3] (6)[3]	0 (8)	0 (7)
	C10-18	32 (53)	19 (38)	2[3] (9)[3]	1 (12)	0 (10)
	E300-3	30 (50)	6[3] (27)[3]	0[3] (5)[3]	0 (7)	0 (5)
2	W5	41 (57)	38 (55)	14 (28)	nd[4]	nd
	A10-9	41 (56)	14[3] (31)[3]	0[3] (7)[3]	nd	nd
	C10-18	41 (57)	36 (49)	18 (14)	nd	nd
3	W5	44 (66)	39 (38)	27 (49)	0 (8)	nd
	E300-3	43 (67)	5[3] (15)[3]	1[3] (9)[3]	0 (2)[3]	nd

[1] Number of fruits, each with 3 inoculation sites, used per treatment in experiment 1, 2 and 3: 2, 4 and 3, respectively. Assessment in experiment 1, 2 and 3: 6, 7 and 7 days after inoculation.
[2] Average diameter of fungal colonies on fruits (mm); figures in parentheses represent diameter of macerated tissue.
[3] Significantly different from W5 at P = 0.05 (Student test).
[4] nd = not determined.

Tabel 2. Effect van curatieve dompelbehandelingen van sinaasappels met geformuleerd fenpropimorf (Corbel) tegen rot veroorzaakt door het wild-type W5 en fenarimol-resistente isolaten van Penicillium italicum.

ficacy of fenpropimorph to isolates W5 (wild-type) and C10-8 (low level of fenarimol-resistance) was almost similar. The efficacy against isolates A10-9 and E300-3 (high level of fenarimol-resistance) was significantly higher than to the wild-type isolate. The result of one of the experiments (no. 3) is illustrated in Fig. 1.

Discussion

The most important groups among the EBI's are imidazole (e.g. imazalil, fenapanil), morpholine (e.g. fenpropimorph, tridemorph), pyrimidine (e.g. fenarimol) and triazole derivatives (e.g. bitertanol, etaconazole). Positively correlated cross-resistance to imidazole, pyrimidine, and triazole derivatives has frequently been reported (cf. De Waard and Fuchs, 1982). The same is true for fenarimol-resistant isolates of *P. italicum* (De Waard et al., 1982). Results of this study show that some of these isolates lack cross-resistance to fenpropimorph. A lack of in vitro cross-resistance to morpholines has also been described for mutants of several other fungi (Barug and Kerkenaar, 1979; Leroux and Gredt, 1981). However, as distinct from the latter observations most of the *Penicillium* isolates tested showed a negatively correlated cross-resistance to fenpropimorph, both in in vitro and in vivo experiments. Such a phenomenon has not been reported previously for other EBI-resistant mutants.

Fig. 1. Effect of curative dip treatments of oranges with formulated fenpropimorph (Corbel) against decay incited by the wild-type W5 and the fenarimol-resistant isolate E300-3 (= E3) of *Penicillium italicum*. Fenpropimorph concentrations: 0, 10, 30 and 100 μg a.i. ml^{-1}.

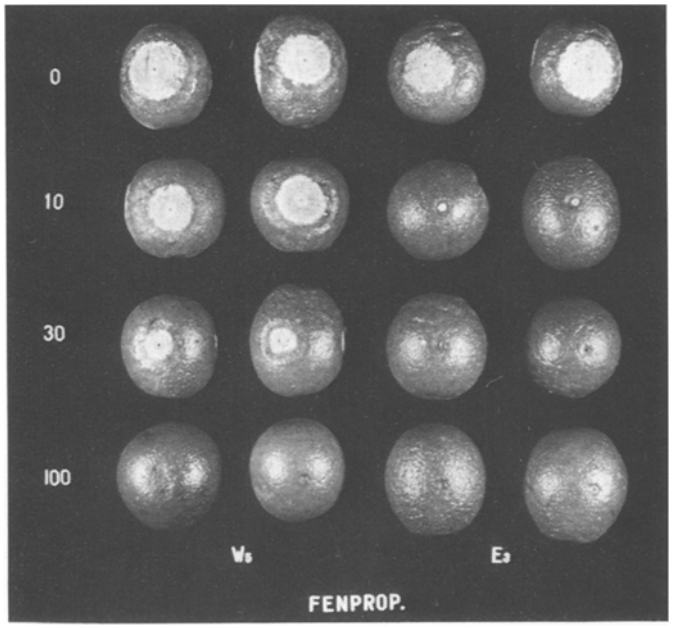

Fig. 1. Effect van curatieve dompelbehandelingen van sinaasappels met geformuleerd fenpropimorf (Corbel) tegen rot veroorzaakt door het wild-type W5 en het fenarimol-resistente isolaat E300-3 (= E3) van Penicillium italicum. Fenpropimorf concentraties: 0, 10, 30 en 100 μg a.i. ml^{-1}.

An explanation for the lack of cross-resistance to different EBI's is not known. The mechanism of resistance to fenarimol in *Aspergillus nidulans* is based on reduced uptake due to enhanced active efflux of the fungicide (De Waard and Van Nistelrooy, 1980). The same mechanism might be operative in *P. italicum* . In that case increased efflux activity for fenarimol might be related with normal or decreased efflux activity for fenpropimorph. Lack of cross-resistance might also be due to the fact that the fungicides inhibit ergosterol biosynthesis at different sites: whereas morpholine derivatives like tridemorph inhibit Δ^8-Δ^7 isomerization (Kato et al., 1980) or Δ^{14}-reductase (Kerkenaar et al., 1981), fenarimol and other EBI's inhibit C-14 demethylation (Buchenauer, 1977). Fenpropimorph is structurally related to tridemorph and most probably acts in a similar way. Therefore, a resistance mechanism based on decreased affinity of the target enzyme for fenarimol might explain lack of cross- resistance to fenpropimorph. Such a mechanism, however, does not explain the negatively correlated cross-resistance to both fungicides.

The practical significance of the normal or increased sensitivity of fenarimol-resistant isolates to fenpropimorph depends on whether or not, in addition, strains with positively correlated cross-resistance do occur in the population. Such strains were not found among the isolates tested. This compares favourably with the situation found

with other chemicals such as the benzimidazoles (Van Tuyl et al., 1974) and carboxamides (White et al., 1978; White and Thorn, 1980). Nevertheless, the number of isolates tested is too low to state categorically that the chance of development of isolates with positively correlated cross-resistance to fenarimol and fenpropimorph is 'very low' or nil. If so, one might consider alternating or combined use of fenpropimorph with fenarimol or related EBI's like imazalil to be effective in reducing or avoiding the risk of development of resistance to EBI's.

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Samenvatting

Toxiciteit van fenpropimorf voor fenarimol-resistente isolaten van Penicillium italicum

Fenarimol-resistente isolaten van *Penicillium italicum* met kruisresistentie tegen imidazool- en triazoolfungiciden die de ergosterolbiosynthese remmen, werden getoetst op hun gevoeligheid voor fenpropimorf. Radiale groeiproeven op PDA toonden aan dat de isolaten (n = 6) geen kruisresistentie bezaten met fenpropimorf of een verhoogde gevoeligheid voor het middel vertoonden (negatief gecorreleerde kruisresistentie). Op sinaasappels kon *Penicillium*-rot, veroorzaakt door het wild-type bestreden worden door middel van een dompelbehandeling met fenpropimorf bij een dosering van 100 μg ml^{-1}). Bestrijding van rot veroorzaakt door fenarimil-resistente isolaten werd verkregen bij dezelfde of een lagere dosering (30 μg ml^{-1}); aldus werd aangetoond dat de normale of verhoogde gevoeligheid voor fenpropimorf ook in vivo tot uiting komt.

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