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Differential accumulation of fenarimol by a wild-type isolate and fenarimol-resistant isolates of Penicillium italicum

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Abstract

Accumulation of [^{14}C] fenarimol by mycelium of *Penicillium italicum* was studied with isolates having varying levels of laboratory resistance to fenarimol. All resistant isolates tested showed a significantly lower accumulation than the wild-type isolate.

Various metabolic inhibitors enhanced accumulation to relatively high levels in both wild-type and resistant isolates. It indicates that accumulation is governed by two processes viz. a non-mediated influx and an energy-dependent efflux. A relatively high fenarimol efflux in resistant isolates probably accounts for low accumulation and for fenarimol resistance. One of the inhibitors which annihilated fenarimol efflux and enhanced fenarimol accumulation was sodium orthovanadate. The synergistic action of fenarimol and orthovanadate to both wild-type and resistant isolates in crossed-paper strip bioassays is probably related to the effect of the latter compound on fenarimol accumulation. Synergistic action between the chemicals in control of *Penicillium* decay of oranges could not be detected.

Additional keywords: ergosterol biosynthesis inhibitors, energy-dependent efflux, synergism.

Introduction

Over the past fifteen years an increasing number of antifungal ergosterol biosynthesis inhibitors (EBIs) has been introduced in crop protection (Schwinn, 1983). Their mechanism of action has recently been reviewed by Langcake et al. (1983) and by Davidse and De Waard (1984). Knowledge on mechanisms of resistance to EBIs is limited. This is probably due to the fact that development of resistance to this type of fungicides under field conditions does not readily occur. It has only been observed in *Sphaerotheca fuliginea* (Schepers, 1983). Selection of laboratory isolates of various fungi with EBI resistance is often quite simple and has been carried out with *Aspergillus nidulans* by De Waard and Gieskes (1977) and Van Tuyl (1977) and with *Penicillium italicum* by De Waard et al. (1982). Resistance in *A. nidulans* is most probably due to constitutive energy-dependent efflux of fenarimol resulting in decreased accumulation of the fungicide (De Waard and Van Nistelrooy, 1979, 1980). Annihilation of ferarimol efflux with respiratory inhibitors or phthalimide fungicides enhanced accumulation and could with some of these chemicals be correlated with increased sensitivity to fenarimol or synergism (De Waard and Van Nistelrooy, 1979, 1980, 1982, 1984). It has been suggested that such effects can be useful in the design of rational approaches in disease control strategies (Fuchs et al., 1983). This would be the more

so if the same mechanism of resistance should occur in pathogenic fungi. For this reason, the mechanism of resistance to fenarimol in *P. italicum* was investigated. The aim of this study was to examine whether resistance in various fenarimol-resistant laboratory isolates could be related with decreased accumulation of the fungicide as described for *A. nidulans*. In addition, the effect of various chemicals on accumulation of fenarimol was studied and experiments were carried out to test whether enhanced accumulation of fenarimol would again result in increased sensitivity to fenarimol or in synergism.

Materials and methods

Fungal strains. The *P. italicum* isolates used were the wild-type W5 and the fenarimol-resistant isolates C10-18 (low degree of resistance), A10-9 and B10-4 (medium degree of resistance) and E300-3 (high degree of resistance). They have been described previously (De Waard et al., 1982).

Chemicals. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) and cetylpyridinium bromide (CPB) were purchased from Sigma Chemical Company (St. Louis, Missouri, USA), dicyclohexylcarbodiimide (DCCD) from Baker Chemicals B.V. (Deventer, the Netherlands and sodium orthovanadate (Na_3VO_4) from Janssen Chimica (Beerse, Belgium). All fungicides were generous gifts; bitertanol and triadimefon from Bayer A.G. (Leverkusen, FRG), captafol and captan from Ligtermoet Chemie B.V. (Roosendaal, the Netherlands), etaconazole from Ciba Geigy Ltd (Basel, Switzerland), fenarimol, [$\alpha-{}^{14}$C]fenarimol (spec. act. 36.4 μCi mmol^{-1}; 1.35 MBq mmol^{-1}, unless otherwise indicated), and Rubigan (12% EC fenarimol) from Lilly Research Centre Ltd (Erl Wood Manor, England), folpet from Ortho Chevron Chemical Company (Paris, France), imazalil from Janssen Pharmaceutica (Beerse, Belgium). All chemicals were of technically pure or crystalline grade.

Culture methods and preparation of mycelial suspension. The *P. italicum* isolates were maintained on malt extract agar (Oxoid CM59). Mycelium was grown in a liquid medium containing 2% malt extract (Oxoid L39) and 0.2% mycological peptone (Oxoid L40). Flasks (300 ml) with medium (100 ml) were inoculated with 1 ml of a conidial suspension (10^9 conidia ml^{-1}) and incubated at 25 °C on a Gallenkamp orbital shaker at 180 rpm for about 16 h. Then, mycelium was harvested by filtration on a Büchner filter and washed twice by resuspending the wet mycelium (1 g) in 23.4 mM potassium phosphate buffer pH 6.0 (50 ml) containing 0.1 mM calcium chloride and 1% (w/v) glucose. Standard mycelial suspensions with an average dry weight of 3.0 mg ml^{-1} were made by resuspending washed mycelium (1 g) in 50 ml of the same medium, unless otherwise indicated.

Accumulation of [^{14}C]fenarimol. Standard mycelial suspensions (50-100 ml) were shaken in a reciprocal shaker at 27 °C for 30 min. Experiments were initiated by adding [^{14}C] fenarimol to a final concentration of 90 μM from a 100-fold concentrated stock solution in methanol. At intervals, mycelium of 5-ml samples was collected on Whatman GF/A glass filter paper, using a Millipore sampling manifold apparatus, and washed five times within 30 s with incubation medium (5 ml). The mycelium was

transferred into scintillation vials and extracted with a mixture of 4 ml Aqualuma Plus (Lumac, Schaesberg, the Netherlands) and 0.5 ml water for at least 40 h. Radioactivity was counted in a liquid scintillation spectrometer. Effects of various chemicals on the accumulation of [^{14}C]fenarimol were determined by addition of 100-fold concentrated stock solutions in methanol or water, 15 min prior to or 65 min after addition of [^{14}C]fenarimol to the standard mycelial suspension. The effect of sodium orthovanadate was tested with mycelium suspended in 25 mM Tris-buffer pH 8.0 containing 0.1 mM calcium chloride and 1% (w/v) glucose since phosphate ions may reduce the inhibitory action of orthovanadate on plasma membrane ATPase activity and inhibition of enzyme activity is more efficient at alkaline pH (Dufour et al., 1980). Orthovanadate was added from a 333-fold concentrated stock solution in water (pH 8). The viability of the mycelium in the various experiments was determined by resuspending washed mycelial residues in sterile water (5 ml), transferring small droplets of these suspensions to malt extract agar, and measuring radial growth after incubation for 2 days at 25 °C.

Crossed-paper strip bioassay. The synergistic action between fenarimol and other chemicals was tested qualitatively using the crossed-paper strip bioassay (De Waard and Van Nistelrooy, 1982). Malt extract agar was used. Conidia were suspended in agar (10^7 ml^{-1}) at 45 °C just before pouring the plates instead of seeding the agar surface.

Toxicity of fenarimol. Toxicity of fenarimol to mycelial growth in liquid media was tested by resuspending mycelium (1 g), washed with water, in malt peptone medium (100 ml) or in a synthetic medium (100 ml) according to Bartz and Eckert (1972). The suspensions were divided into 50-ml portions in flasks (300 ml) and incubated under growth conditions. Fenarimol was added after 30 min of preincubation from a 100-fold concentrated stock solution in methanol. Growth was measured in triplicate by determining increase in mycelial dry weight after 6 h of incubation.

The toxicity of combinations of fenarimol and sodium orthovanadate was tested in malt peptone liquid medium using conidia as inoculum as described under 'Culture methods'. Mycelial growth was measured in triplicate as dry weight increase during a 16-h incubation period.

The methods used for determining the efficacy of chemicals in controlling *Penicillium* decay upon dip treatment of oranges has been described previously (De Waard et al., 1982).

Results

Toxicity of fenarimol. Growth inhibition of *P. italicum* W5 at 30, 100 and 300 μM fenarimol in the malt peptone medium was 4, 17 and 34%, respectively. During the 6-h growth period dry weight in control treatments increased from 1.7 to 4.6 mg ml^{-1}. Under similar conditions growth inhibition in the synthetic medium was 4, 2 and 15%, respectively. Dry weight increased from 1.5 to 3.0 mg ml^{-1}. The EC_{50} value of fenarimol for inhibition of radial growth on PDA or malt agar is 0.9 μM (De Waard et al., 1982). Hence, toxicity of fenarimol to mycelial growth in liquid cultures under the conditions used was significantly lower than for radial growth on PDA. This is probably due to the high mycelial densities and the relatively short incubation periods used in the growth tests with liquid cultures.

Accumulation of [^{14}C] fenarimol. Introductory experiments were carried out in order to establish optimal conditions for accumulation experiments. Accumulation by mycelium of *P. italicum* W5 and E300-3 was tested at 30, 90 and 270 μM [^{14}C] fenarimol (spec. act. 4.05, 1.35 and 0.45 MBq mmol^{-1}, respectively). The differential accumulation observed (Fig. 1) was most pronounced at 90 μM fenarimol. At 270 μM fenarimol, accumulation by isolate W5 was still higher than by isolate E300-3 but figures were erratic and are, therefore, not presented. Accumulation of [^{14}C]fenarimol (90 μM) was also studied at 1, 1/2, 1/4 and 1/8 times the standard mycelial density. In all cases the differential accumulation patterns by isolate W5 and E300-3 were essentially comparable with those presented in Fig. 1. The only difference observed regarded the level of accumulation, being higher at lower densities: accumulation after 10 min of incubation at 1, 1/2, 1/4 and 1/8 rates amounted to 3.0, 3.4, 4.1 and 4.7 nmol mg^{-1} dry weight of W5 mycelium and 0.4, 0.8, 1.1 and 1.2 nmol mg^{-1} dry weight of E300-3 mycelium, respectively.

At standard mycelial densities the standard length of the preincubation period (30 min) before addition of [^{14}C]fenarimol proved to be critical, since periods of 60 min or longer resulted in a lower and a less pronounced transient accumulation by W5 in time (results not shown). The results described led to the adoption of the standard [^{14}C]fenarimol accumulation assay.

Accumulation of [^{14}C]fenarimol by *P. italicum* W5, A10-9, B10-4, C10-18 and E300-3 according to the standard procedure was compared in two experiments. All results indicated a differential accumulation by the various isolates (Fig. 2). The wild-type showed a transient accumulation, while accumulation by all isolates with a

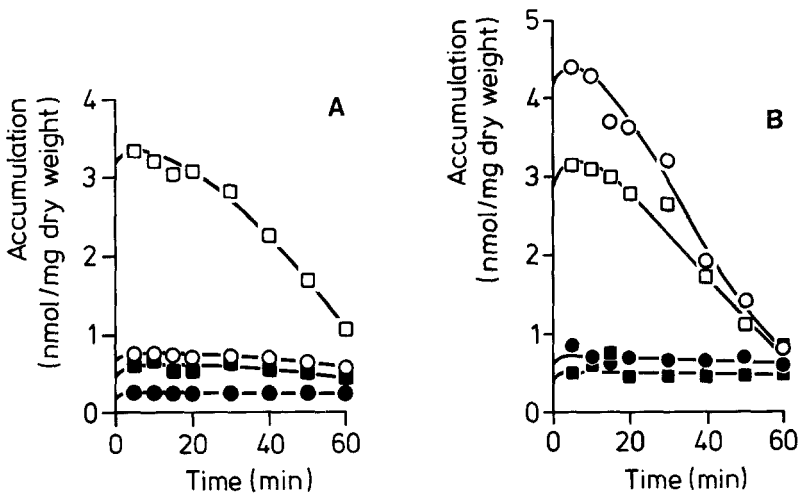

Fig. 1. (A). Accumulation of [^{14}C]fenarimol by the wild-type isolate W5 (○, □) and the fenarimol-resistant isolate E300-3 (●, ■) of *Penicillium italicum*. Concentration of fenarimol 30 μM (○, ●) or 90 μM (□, ■).

Fig. 2. (B). Accumulation of [^{14}C]fenarimol (90 μM) by the wild-type isolate W5 (○) and the fenarimol-resistant isolates C10-18 (□), A10-9 or B10-4 (●) and E300-3 (■) of *Penicillium italicum*.

medium or high degree of fenarimol-resistance (A10-9, B10-4 and E300-3) was relatively low and constant in time. The accumulation pattern by isolate C10-18 with a relatively low degree of resistance was intermediate in both experiments.

Effect of various chemicals on [^{14}C]fenarimol accumulation. Various chemicals were tested with respect to their effect on [^{14}C]fenarimol accumulation by *Penicillium* mycelium when added 65 min after addition of the fungicide. The chemicals included CCCP (uncoupler of mitochondrial ATP synthesis), DCCD (inhibitor of ATPase activity), the phthalimide fungicides captan, captafol and folpet (sulfhydryl reagents), sodium orthovanadate (inhibitor of plasma membrane ATPase activity), CPB (cationic detergent) and copper sulphate (a.o. a fungitoxic agent). All these chemicals instantaneously increased accumulation (Table 1; Fig. 3). Most pronounced effects were observed with the latter two chemicals mentioned. In the case of CPB a level of almost 15 nmol mg^{-1} dry weight was reached. This means that under these conditions about 50% of the [^{14}C]fenarimol added accumulated in mycelium (calculation based on an average mycelial dry weight of 3 mg ml^{-1}). Mycelium always survived the various treatments, except those with CPB.

Effect of other EBIs on [^{14}C]fenarimol accumulation. Preincubation of *A. nidulans* (wild-type) with EBIs for 90 min prevented transient accumulation of fenarimol (De Waard and Van Nistelrooy, 1982). The EBI fungicides etaconazole, imazalil and triadimefon were tested for a similar potency in *P. italicum* W5. Since relatively long preincubation periods influenced the efflux of [^{14}C]fenarimol by mycelium of isolate

Table 1. Effect of various chemicals on accumulation of [^{14}C]fenarimol (90 μM) by *Penicillium italicum* wild-type isolate W5 and fenarimol-resistant isolate E300-3.

Exp.	Treatment[2] and concentration	Accumulation (nmol mg^{-1} dry weight)							
		W5				E300-3			
		t=10	t=60	t=75	t=125	t=10	t=60	t=75	t=125
I	Control	2.7	1.1	1.2	1.0	0.5	0.4	0.5	0.5
	CCCP (100 μM)	–	–	3.4	2.9	–	–	3.1	3.4
	DCCP (100 μM)	–	–	2.6	1.9	–	–	1.3	1.4
II	Control	4.3	1.4	0.8	0.6	0.5	0.5	0.5	0.5
	Captan (100 μM)	–	–	2.8	3.8	–	–	2.5	4.1
	Captafol (100 μM)	–	–	4.0	3.5	–	–	3.5	3.7
	Folpet (100 μM	–	–	2.6	2.8	–	–	2.2	3.2
III[1]	Control	4.4	0.5	0.4	0.4	0.4	0.4	0.4	0.4
	Na_3VO_4 (30 mM)	–	–	2.2	1.7	–	–	2.2	1.6
IV[1]	Control	3.9	0.8	0.4	0.4	0.4	0.5	0.5	0.5
	CPB (2.6 mM)	–	–	14.9	13.1	–	–	14.8	12.0
	$CuSO_4$ (7.0 mM)	–	–	3.9	9.8	–	–	3.2	9.1

[1] Average of two experiments.
[2] Test chemicals added 65 min after addition of [^{14}C]fenarimol (t = 65).

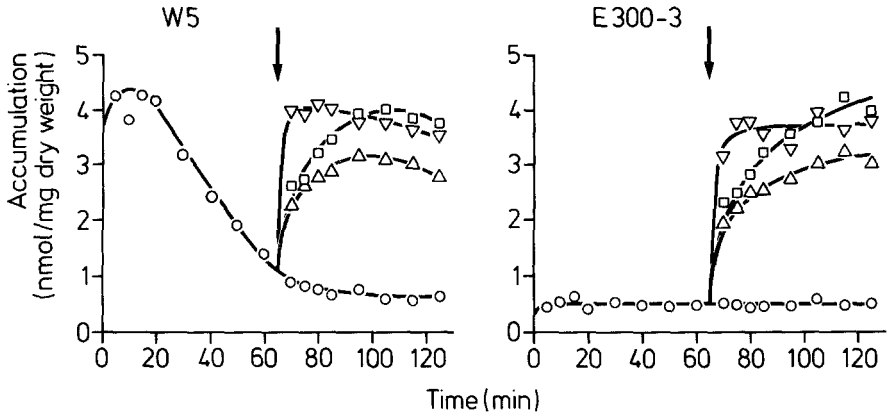

Fig. 3. Effect of the phthalimide fungicides captan (□), captafol (▽) and folpet (△) on accumulation of [^{14}C]fenarimol (90 μM) by the wild-type isolate W5 and the fenarimol-resistant isolate E300-3 of *Penicillium italicum*. Control treatment (○). The phthalimides were added 65 min after the addition of [^{14}C]fenarimol (marked with arrow).

Table 2. Effect of preincubation of *Penicillium italicum* wild-type W5 with etaconazole, imazalil and triadimefon (270 μM) on accumulation of [^{14}C]fenarimol (90 μM).

Fungicide and time of addition[1]		Accumulation (nmol mg^{-1} dry weight)			
		t = 5	t = 20	t = 40	t = 60
Etaconazole	control	4.2	4.6	3.5	1.6
	t = 0	6.3	5.8	4.5	2.2
	t = −30	4.3	2.4	1.2	0.8
Imazalil	control	4.5	4.3	2.9	1.1
	t = 0	5.6	5.5	4.5	3.4
	t = −30	4.6	1.9	1.0	0.7
Triadimefon	control	5.6	5.1	3.2	0.8
	t = 0	6.7	6.8	6.1	4.8
	t = −30	4.5	2.4	1.4	1.0

[1] Each experiment consisted of three treatments: a control with only [^{14}C]fenarimol, a treatment with [^{14}C]fenarimol and the test fungicide added simultaneously (t = 0), and a treatment with the test fungicide added 30 min prior to addition of [^{14}C] fenarimol (t = −30).

W5, this period had to be reduced to 30 min. The data (Table 2) indicate that preincubation with the EBIs at 270 μM resulted in a reduced accumulation of [^{14}C]fenarimol. The reduction was most evident after 20 and 40 min of incubation. Simultaneous addition of the test fungicides with [^{14}C]fenarimol enhanced accumulation of the latter chemical. At 90 μM all test chemicals showed an intermediate effect (data not shown).

Synergistic activity. A number of chemicals which enhanced [^{14}C]fenarimol accumulation by *A. nidulans* showed synergistic action with the fungicide in crossed-paper strip bioassays (De Waard and Van Nistelrooy, 1982). Therefore, the chemicals mentioned in Table 1 were also tested for synergistic action with fenarimol to *P. italicum*. None of them responded positively, except for sodium orthovanadate (Fig. 4). Synergism with this chemical was most evident with the resistant isolate, since in this case neither the test chemical itself, nor fenarimol were inhibitory to fungal growth. Comparable results were obtained with the other resistant isolates A10-9 and C10-18. Combinations of paper strips impregnated with orthovanadate and other EBI fungicides (a.o. bitertanol, etaconazole and imazalil) in bioassays with isolates W5 and E300-3 also resulted in similar synergistic actions as described above for fenarimol (results not presented).

Quantitative determination of synergism was carried out in liquid medium (Table 3). At the fenarimol concentrations used, growth of the wild-type and the resistant isolate was reduced to 66 and 53% of the untreated controls, respectively. In the presence of orthovanadate these figure were only slightly lower, indicating only a marginal synergistic interaction under these test conditions.

Synergistic action between fenarimol and orthovanadate in the control of *Penicillium* rot of oranges was also examined. Oranges were soaked during one night in a sodium orthovanadate solution (30 mM), inoculated with isolate E300-3, and incubated for 1 day at 25 °C. Then they were dip-treated in a suspension of Rubigan (30 mM fenarimol). The fenarimol treatment alone effectively controlled decay incited by the wild-type but failed to control the disease when caused by isolate E300-3 (De Waard et al., 1982). Orthovanadate treatment alone had no obvious effect on disease

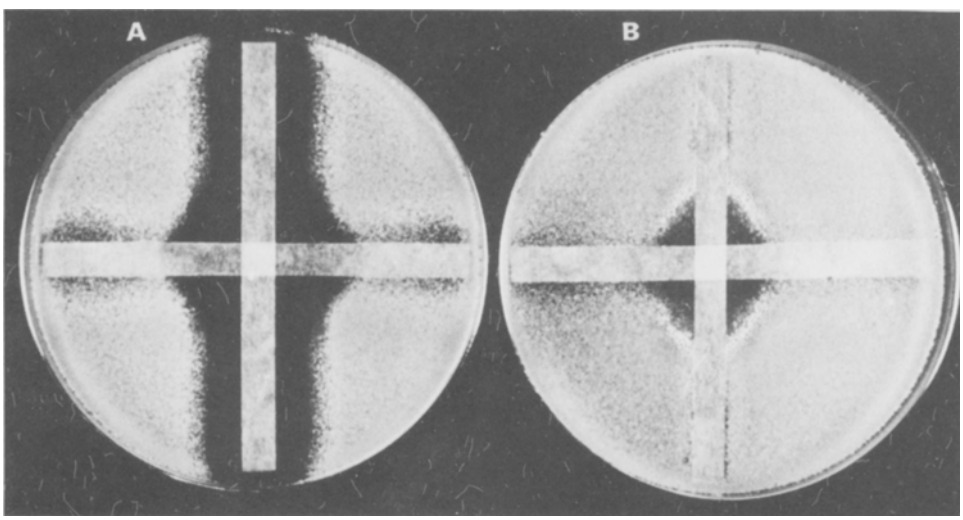

Fig. 4. Interaction between the toxicities of sodium orthovanadate and fenarimol to the wild-type isolate W5 (A) and the fenarimol-resistant isolate E300-3 (B) of *Penicillium italicum* in crossed-paper strip bioassays. Horizontal strips treated with sodium orthovanadate (1 M solution in water pH 8.0) were placed onto agar 16 h before vertical strips with fenarimol (3 mM solution in methanol).

Table 3. Interaction between the toxicities of sodium orthovanadate and fenarimol to mycelial growth of *Penicillium italicum* W5 and E300-3 in a liquid malt peptone medium.

Sodium orthovanadate concentration	Isolate W5			Isolate E300-3		
	growth (mg ml^{-1})		percentage of control	growth (mg ml^{-1})		percentage
	control	fenarimol[1]		control	fenarimol[1]	
0	4.04 ± 0.05	2.65 ± 0.11	66	3.91 ± 0.06	2.07 ± 0	53
30 mM	3.04 ± 0.17	1.47 ± 0.04	48	3.01 ± 0.02	1.34 ± 0.05	45

[1] Concentration of fenarimol in experiment with isolates W5 and E300-3, 1.5 and 30 μM, respectively.

incidence, but did have phytotoxic effects on the peel of the fruits. Combined treatments did not slow down development of *Penicillium* decay as compared with the control fenarimol treatment (results not presented). Hence, no obvious synergism was detected.

Discussion

Accumulation of fenarimol by *P. italicum* was studied at concentrations of the fungicide that are sublethal for the wild-type W5. Accumulation by all fenarimol-resistant isolates tested was always lower than by the wild-type isolate. It was most evident at a fenarimol concentration of 90 μM (Fig. 1). Accumulation by the wild-type W5 was relatively high but transient and by the resistant isolates A10-9, B10-9 and E300-3 constantly low. Isolate C10-18, which has the lowest degree of resistance, showed intermediate results (Fig. 2). A consequence of such a differential accumulation can be that in resistant isolates the fungicide does not accumulate to toxic concentrations at its target site in the ergosterol biosynthetic pathway. It may, therefore, account for the mechanism of resistance to fenarimol in *P. italicum*. A similar mechanism has been described previously in *A. nidulans* (De Waard and Van Nistelrooy, 1979, 1980).

The effect of various chemicals on fenarimol accumulation was tested in order to characterize the processes involved (Table 1). Incubation with the respiratory inhibitors CCCP and DCCD resulted in enhanced accumulation by both wild-type and resistant isolates. Phthalimide fungicides had a similar effect. Comparable results have been described for *A. nidulans* (De Waard and Van Nistelrooy, 1980, 1984). The enhancement can be accounted for by assuming that accumulation of fenarimol is the result of passive, non-mediated influx and energy-dependent efflux. It is probable that the efflux has to be activated in the wild-type but is constitutive in resistant isolates except for isolate C10-18. The nature of the fenarimol efflux is not yet certain. The efflux can also be inhibited by sodium orthovanadate (Table 1), an inhibitor of fungal plasma membrane ATPase but not of mitochondrial ATPase (Goffeau and Slayman, 1981). The enzyme mediates transport of many natural substrates. It is, therefore, supposed that the enzyme or rather the electrochemical proton gradient created by the enzyme over the plasma membrane, also mediates fenarimol efflux. In this concept ac-

cumulation of fenarimol is inversely related to the magnitude of the electrochemical proton gradient or the energized state of the membrane. A low energized state is, among others, attained upon uncoupling of mitochondrial ATP synthesis (CCCP) or by inhibition of plasma membrane ATPase activity (orthovanadate) and would result in high levels of fenarimol accumulation. In the wild-type isolate fenarimol itself or other EBIs (Table 2) may enhance the energized state of plasma membranes as a consequence of which accumulated fenarimol is gradually released. Similarly, a permanently high energized state of the plasma membrane in resistant isolates may also effect low accumulation.

Incubation of mycelium with the cationic chemicals CPB or copper sulphate resulted in extremely high levels of fenarimol accumulation (Table 1) With CPB about 50% of the amount of fungicide added had accumulated. This might have been due to a highly effective dissipation of the plasma membrane potential which together with the pH gradient over the plasma membrane composes the electrochemical proton gradient.

Chemicals which enhance mycelial accumulation of fenarimol may have a synergistic action on fungal toxicity. In crossed-paper strip bioassays it was only observed with sodium orthovanadate, especially with the resistant isolate E300-3 (Fig. 1). It is unclear why combination of fenarimol with CCCP, DCCD or phthalimide fungicides showed no effect, while these combinations were synergistic with *A. nidulans* (De Waard and Van Nistelrooy, 1982, 1984). Combinations of orthovanadate and other EBIs were also synergistic. Such results emphasize the importance of further search for other synergistic chemicals which may be useful under practical conditions.

Experiments with fenarimol and orthovanadate on control of *Penicillium* decay of oranges did not show a synergistic interaction. The lack of a synergistic interaction may be due to a variety of factors as for instance spatial separation of the chemicals over the oranges. Nevertheless, the improved performance of recommended mixtures of EBIs with conventional fungicides in practice may be due to the phenomenon described above (Fuchs et al., 1983). It has, for instance, been observed for a mixture of fenpropimorph and chlorothalonil which effectively reduced disease incidence of *Pyrenophora teres* in barley. The individual components of this mixture had only slight activities (Hampel and Lartaud, 1983). It would be interesting to investigate whether such a synergistic action can be explained by enhanced accumulation of fenpropimorph in the presence of the other fungicide.

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Samenvatting

Differentiële accumulatie van fenarimol door een wild-type isolaat en fenarimol-resistente isolaten van Penicillium italicum

De accumulatie van [^{14}C]fenarimol door mycelium van *Penicillium italicum* werd bestudeerd bij isolaten met een uiteenlopende graad van laboratorium-resistentie

tegen fenarimol. Alle getoetste resistente isolaten vertoonden een lagere opname dan de wild-stam.

Verschillende antimetabolieten verhoogden de accumulatie tot relatief hoge waarden die voor gevoelige en resistente isolaten ongeveer gelijk waren. Deze waarneming duidt erop dat accumulatie wordt bepaald door twee processen: 'non-mediated' influx en energie-afhankelijke efflux. Een hogere fenarimol efflux in resistente isolaten vormt waarschijnlijk de verklaring voor de lagere accumulatie en voor het resistentiemechanisme. Een van de remmers die de fenarimolefflux te niet doet, en de accumulatie van fenarimol verhoogt, is natriumorthovanadaat. De synergistische werking van fenarimol en orthovanadaat tegen zowel wild-type als resistente isolaten in 'crossed-paper strip' biotoetsen houdt waarschijnlijk verband met het effect van laatstgenoemde stof op de accumulatie van fenarimol. Synergistische werking van deze verbindingen bij de bestrijding van *Penicillium*-rot op sinaasappels werd niet waargenomen.

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Book review

E. Kurstak (Ed.), 1982. Microbial and viral pesticides. Marcel Dekker AG Publishers, Basel. 720 pp. With tables, illustrations, references at the end of each contribution, 10 pages of general index. Cloth bound. Price SFr. 284.

The problems associated with the wide-spread use of chemical insecticides (environmental pollution, resistance and secondary pests) have stimulated studies on alternative control strategies that are ecologically sound. Although chemical insecticides are essential to maintaining the present level of agricultural production, alternative pest control strategies based on integration of different methods and agents, including the use of microbial and viral pesticides, have received more attention during the last decade. This is due to successful release of some of these biological control agents and the notable progress in basic research at the molecular level. The purpose of this treatise 'Microbial and viral pesticides' is to present a comprehensive review of the literature on practical applications and the safety of microbial and viral agents, and to give an update on recent developments in this area.

In this volume, the editor has brought together 21 chapters from various experts, and divided these chapters among 7 sections. The book is of considerable size (720 pp.) and includes over a thousand references. After a general introduction (section I), the relevant groups of pathogens with pest control potential, i.e. bacteria, viruses, fungi and protozoa, are the subject of the following sections (II through V). A separate section (VI) is devoted to microbial herbicides. The varying size of the different sections reflects the relative potential and importance of these agents in insect and weed control. The book concludes with a section on registration of microbial and viral pesticides (section VII).

In the rather scanty general introduction (section I), the mode of action, safety aspects, and 'future prospects' of microbial and viral pesticides are discussed, and the authors (E. Kurstak