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Simultaneous resistance in fungi to ergosterol biosynthesis inhibitors and dicarboximides

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Abstract

Several strains of *Aspergillus nidulans, Cladosporium cucumerinum* and *Penicillium italicum* with known resistance to ergosterol biosynthesis inhibitors were tested for resistance to three dicarboximides. Negligible levels of resistance to iprodione and vinclozolin were observed in one out of three strains of *A. nidulans.* Two out of three strains of *C. cucumerinum* displayed a low resistance to iprodione, and a high resistance to procymidone and vinclozolin. The latter strains were also moderately resistant to the isoflavonoid phytoalexins medicarpin and pisatin, but sensitive to the antibiotic pimaricin. All six *P. italicum* strains examined displayed wild-type sensitivity to all three dicarboximides; the two of these tested in thin-layer chromatographic bioassays proved to be resistant to pimaricin.

Iprodione and vinclozolin induced energy-dependent fenarimol efflux in *A. nidulans.* In line with this observation, in crossed-paper strip assays iprodione and fenarimol antagonized each other in their toxicity towards *A. nidulans;* towards *C. cucumerinum*, on the other hand, these fungicides behaved independently.

The implications and practical consequences of the phenomena observed are briefly discussed.

Introduction

Fungal strains with in vitro resistance to ergosterol biosynthesis inhibitors (EBI's) can be readily obtained *(cf.* Fuchs and De Waard, 1982, and De Waard and Fuchs, 1982). There are also many reports of in vitro resistance to dicarboximide fungicides *(cf.* Beever and Byrde, 1982).

As the name implies, EBI's interfere with ergosterol biosynthesis, mostly by inhibiting C-14 demethylation, but in some cases by blocking $\Delta^8 \rightarrow \Delta^7$ isomerization (Kato et al., 1980) or by affecting 14/15 double-bond reduction (Kerkenaar et al., 1981). The mode of action of dicarboximide fungicides, on the other hand, is still largely unknown. Several target sites have been proposed (*cf.* Beever and Byrde, 1982); for instance, iprodione has been suggested to interfere with ergosterol biosynthesis; the accumulation of 4,4-demethylsterols indicates that it inhibits at a site most probably different from that of C-14 demethylation (Pappas and Fisher, 1979). Controversial data on fungal resistance to this fungicide can in many cases be accounted for by its structural rearrangement to a much less active isomer in the (m)ethanolic solutions often used in its biological testing (Cooke et al., 1979; *cf.* Beever and Byrde, 1982, and Fuchs and De Waard, 1982).

Because of the alleged interference with sterol biosynthesis by iprodione, it was of interest to investigate whether strains of fungal species known to be resistant to EBI's displayed cross-resistance to dicarboximides. Widespread cross-resistance between these two different groups of fungicides would, indeed, be of practical consequence, since in that case simultaneous or sequential use of representatives of both groups might create potential hazards to the performance of those fungicides among them, to which resistance has not emerged so far.

In this paper a more extensive report is given of results presented at a symposium on 'Resistance to fungicides in plant pathogens' (Fuchs and De Waard, 1981).

Materials and methods

Fungal strains. The origin of the *Aspergillus nidulans* strains (one wild type and three resistant strains) has been described before (De Waard and Gieskes, 1977). Resistance in the latter strains is due to the so-called *imaB* gene for resistance; in addition, strain R 264 carries the *imaA* gene and a gene which amplifies its action (Van Tuyl, 1977).

Of the four *Cladosporium cucumerinum* strains used (one wild type and three resistant strains) the wild type and Ta 22 have been described elsewhere (Fuchs and Viets-Verweij, 1975); Ta 22 was obtained after UV-irradiation of spore suspensions of the wild-type strain by growing it in the presence of triarimol as selective agent. U6-1000 and W 1-0 were both kindly provided by Dr I.F. Brown (Lilly Research Laboratories, Greenfield, Indiana, USA). U6-1000 gradually lost its initial high degree of fenarimol resistance (minimum inhibitory concentration >1000 $\mu g.ml^{-1}$) upon routine subculturing on fungicide-free media.

The seven *Penicillium italicum* strains (one wild type and six resistant strains) all originate from earlier work on laboratory resistance to EBI's in this species (De Waard et al., 1982). With all resistant strains, the selective agent used was fenarimol.

Mycelial growth tests; thin-layer chromatographic bioassays. Mycelial growth tests of wild types and resistant strains were carried out using either malt (Oxoid) or potato dextrose (Merck) agar. The dosage-response curves obtained provided the EC_{50}-values for inhibition of mycelial growth. TLC-bioassays were performed according to Homans and Fuchs (1970), using *n*-hexane/ethyl acetate/methanol (60 : 40 : 1) as the solvent system. Degrees of resistance were expressed as the ratio EC_{50} resistant strain/EC_{50} wild type as described previously (Fuchs et al., 1977), or based on the size of inhibition zones, relative to those found with wild-type strains, as revealed in TLC-bioassays.

Induction of [^{14}C]-fenarimol efflux by iprodione and vinclozolin. Experiments on fenarimol efflux from mycelial suspensions of the wild-type strain of *A. nidulans* were conducted as described before (De Waard and Van Nistelrooy, 1981). Efflux was induced by incubating mycelium with each of the two test fungicides at a concentration of 300 μM for 90 min before addition of [^{14}C]-fenarimol. Effects of carbonyl cyanide, *m*-chlorophenylhydrazone (CCCP; 0.1 mM) and sodium lauryl sulphate (SLS; 10 mM) on efflux activity were studied by adding these chemicals 65 and 105 min, respectively, after addition of [^{14}C]-fenarimol.

Demonstration of interaction between fenarimol and iprodione. Interaction between fenarimol and iprodione with regard to fungitoxic activity towards *A. nidulans* and *C. cucumerinum* was examined with the crossed-paper strip technique (De Waard and Van Nistelrooy, 1982). All possible combinations of filter paper strips treated with iprodione (3 mM solution) and fenarimol (3 mM solution), applied either simultaneously or with a 10-h interval, were tested. Fungal growth was assessed after 3 days of incubation at 37 °C *(A. nidulans)* or 20 °C *(C. cucumerinum).*

Chemicals. The fungicides used were generously supplied by the firms indicated: (EBI's) fenarimol and [^{14}C]-fenarimol – Lilly Research Centre (Erl Wood Manor, UK); imazalil – Janssen Pharmaceutica (Beerse, Belgium); prochloraz – FBC Ltd (Nottingham, UK); triadimefon – Bayer AG (Leverkusen, Fed. Rep. Germany); triforine – Celamerck (Ingelheim, Fed. Rep. Germany); (dicarboximides) iprodione – Rhône-Poulenc-Phytosanitaire (Lyon, France); procymidone – Sumitomo Chemical Co. Ltd (Osaka, Japan); and vinclozolin – BASF AG (Limburgerhof, Fed. Rep. Germany). Carbonyl cyanide, *m*-chlorophenylhydrazone (CCCP) and sodium lauryl sulphate (SLS) were both obtained from Sigma Chemical Co. (St. Louis, Missouri, USA).

Results

The results on fungicide resistance as observed in mycelial growth tests are summarized in Table 1. Of the three EBI-resistant *A. nidulans* strains the two (J146 and R264) with a high degree of resistance to imazalil proved as sensitive to the dicarboximides as the wild-type strain, the Q-values being <2, which is considered to imply non-resistance (= NR). M193, with the lowest degree of resistance to EBI's, displayed a low level of resistance towards iprodione and vinclozolin. However, as is evident from the dosage-response curves the degree of resistance measured depends upon the EC-values chosen, as exemplified for iprodione (Fig. 1); based on EC_{90}-values the level of resistance of all resistant strains is distinctly higher than if based on EC_{50}-values.

All three resistant strains of *C. cucumerinum* were highly resistant to the EBI's, in particular to fenarimol and triadimefon. Whereas Ta 22 was as sensitive as the wild-type strain towards the dicarboximides, both U6-1000 and W 1-0 displayed a distinct level of resistance to the latter fungicides. With respect to procymidone, the wild-type strain and Ta 22 behaved quite anomalously: inhibition of fungal growth increased with the procymidone concentration, to a maximum between 10 and 25 μg ml^{-1} and then decreased again (Fig. 2). Growth of U6-1000 and W 1-0, on the other hand, was uninhibited at all concentrations tested.

All resistant strains of *P. italicum* equalled each other in their response to both EBI's and dicarboximides; towards triadimefon all these strains were as insensitive and towards the dicarboximides as sensitive as the wild-type strain.

The results of the TLC-bioassays, as presented in Table 2, confirmed those of the mycelial growth tests. Whereas both EBI-resistant strains of each species tested showed varying levels of resistance to the EBI's, all of them were as sensitive to the dicarboximides as their respective wild-type strains, with the notable exception of *C. cucumerinum* W 1-0 which was highly resistant to procymidone and vinclozolin; again, the behaviour of the wild-type strain and Ta 22 towards procymidone was quite

Table 1. Degree of resistance to ergosterol biosynthesis inhibitors and to dicarboximides, based on Q-values (EC_{50} resistant strain/EC_{50} wild type) in mycelial growth tests of several strains of *Aspergillus nidulans, Cladosporium cucumerinum* and *Penicillium italicum,* after 3, 5 and 3 days of incubation, respectively; NR = Q < 2, considered to be non-resistant. Numbers in italics refer to EC_{50}-values (in $\mu g\ ml^{-1}$) of wild-type strains.

Fungal strains	Q-values					
	fen[1]	ima	tri	ipr	pro	vin
A. nidulans (malt agar)	*8.0 (4.0)*[2]	*(0.14)*	*(14)*	*2.1*	*2.9*	*1.4*
J 146 (ima)[1]	3 (5)	(11)	(4)	NR	NR	NR
M 193 (fen)	4 (5)	(4)	(3)	4	NR	2
R 264 (ima)	5 (6)	(57)	(4)	NR	NR	NR
C. cucumerinum (potato dextrose agar)	*0.25*	*0.70*	*0.70*	*0.90*	*5.2*[3]	*2.0*
Ta 22 (triarimol)	15	4	26	NR	NR[3]	NR
U 6-1000 (fen)[4]	200	20	>143	8	>19	>50
W 1-0 (fen)[4]	120	21	>143	3	>19	>50
P. italicum (potato dextrose agar)	*0.30*	*0.21*	*>500*	*1.3*	*2.4*	*1.0*
A 10-9 (fen)	65	3	n.a.[5]	NR	NR	NR
B 10-4 (fen)	75	2	n.a.	NR	NR	NR
C 10-8 (fen)	18	2	n.a.	NR	NR	NR
D 100-4 (fen)	75	3	n.a.	NR	NR	NR
E 300-3 (fen)	135	3	n.a.	NR	NR	NR
E 300-5 (fen)	135	4	n.a.	NR	NR	NR

[1] Fungicides tested were: *fen*arimol, *ima*zalil, *tri*adimefon, *ipr*odione, *pro*cymidone and *vin*clozolin; fungicides used in selection of strains indicated in brackets.

[2] Numbers in brackets refer to results of tests on synthetic medium *(cf.* De Waard & Gieskes, 1977).

[3] For anomalous behaviour of wild-type strain and Ta 22 towards procymidone see Fig. 2.

[4] For these strains no exact Q-values for triadimefon, procymidone and vinclozolin can be given, because EC_{50}'s were $>100\ \mu g\ ml^{-1}$, this concentration being the highest tested.

[5] n.a., not applicable; EC_{50}-values for all strains, wild type included, $>500\ \mu g\ ml^{-1}$ (highest concentration tested).

anomalous (see Table 2, note 4). Remarkably, the latter strain displayed also some resistance to the isoflavonoid phytoalexins medicarpin and pisatin, with degrees of resistance of 1 and 2, respectively. As distinct from the other species, both resistant strains of *P. italicum* tested (A 10-9, E 300-5) showed an even higher level of resistance (3) to pimaricin (results of the latter experiments not given).

Iprodione, and to a lesser extent also vinclozolin, both induced fenarimol efflux in

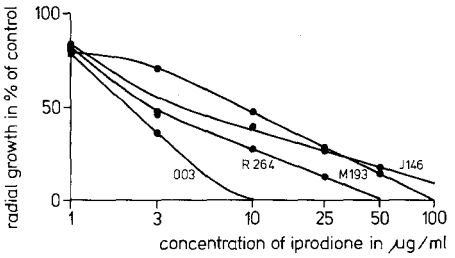

Fig. 1. Dosage-response curves for mycelial growth of four *Aspergillus nidulans* strains (one wild type and three resistant ones) in the presence of iprodione.

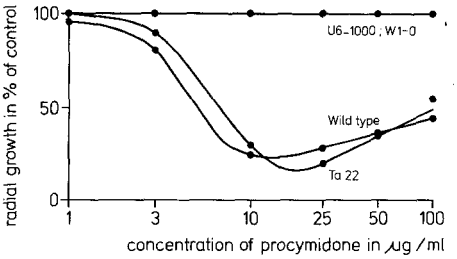

Fig. 2. Dosage-response curves for mycelial growth of four *Cladosporium cucumerinum* strains (one wild type and three resistant ones) in the presence of procymidone. Note the anomalous curves for the wild-type and Ta 22 strains.

Table 2. Degree of fungicide resistance based on size of inhibition zones, relative to those found with wild-type strains, in TLC-bioassays of some strains of *Aspergillus nidulans, Cladosporium cucumerinum* and *Penicillium italicum*. Degree of resistance given in a scale from 0 (no resistance; size of inhibition zone as large as that of wild-type strain) to 5 (complete resistance; no inhibition zone at all).

Fungal strains	fen[1]	prz	tri	tre	ipr	pro	vin
A. nidulans							
J 146	2	1	2	–[2]	0-1	0	0-1
R 264	2	2	3	–[2]	0	0	0
C. cucumerinum							
Ta 22	3	2	1-2	5	0	(0)[3]	0
W 1-0	5	(3)[4]	(2)[4]	5	(1)[4]	5	5
P. italicum							
A 10-9	3	2	0[5]	4-5	0	0	0
E 300-5	3	1	0[5]	4-5	0	0	0

[1] Fungicides tested were: *fen*arimol, *pro*chloraz, *tri*adimefon, *tri*forine, *ipro*dione, *pro*cymidone and *vin*clozolin.

[2] All strains of *A. nidulans* (wild type included) insensitive to triforine.

[3] The behaviour of *C. cucumerinum* towards procymidone was anomalous in that wild-type strain and Ta 22 showed an inhibition zone with fungal growth in the centre of it; W 1-0 showed no inhibition zone at all.

[4] Instead of a zone of inhibition without any fungal growth, a zone of strongly diminished growth was observed.

[5] Very small inhibition zone with all strains of *P. italicum* (wild type included).

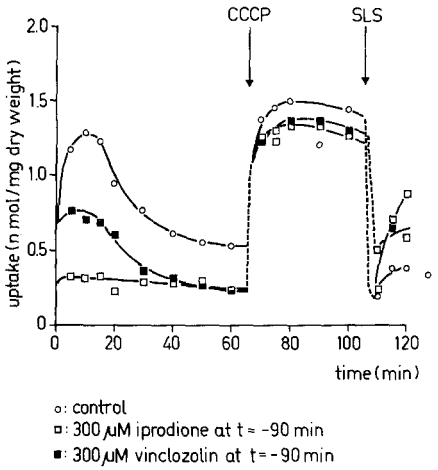

Fig. 3. Effect of iprodione and vinclozolin on uptake of [^{14}C]-fenarimol by the wild-type strain of *Aspergillus nidulans,* and effects of subsequent addition of CCCP and SLS.

the wild-type strain of *A. nidulans* (Fig. 3). This efflux reacted to addition of CCCP and SLS in the same way as when induced by EBI's (De Waard and Van Nistelrooy, 1981).

Additional evidence for fenarimol efflux-inducing activity of iprodione was obtained from experiments with crossed-paper strips: iprodione strongly antagonized fenarimol toxicity towards *A. nidulans,* irrespective of whether the iprodione strip was applied simultaneously with or 10 h before application of the fenarimol strip. Interestingly, fenarimol in its turn antagonized the toxicity of iprodione, against independent of the time interval (0 or 10 h) between applications. Towards *C. cucumerinum,* on the other hand, the two fungicides behaved independently.

Discussion

In interpreting the results of our experiments it should be realized that all EBI's examined most probably have at least one mechanism and site of action in common, since all of them belong to those chemical classes which primarily inhibit C-14 demethylation (cf. Fuchs and De Waard, 1982). In addition, studies with *A. nidulans* (Bellincampi et al., 1980) have shown that EBI's, among which fenarimol, – as well as pimaricin – might exert secondary effects, in causing mitotic non-disjunction. The mode of action of dicarboximides, on the other hand, remains elusive (Beever and Byrde, 1982), though also here induction of mitotic instability has been suggested as a possible, though probably secondary, effect (Georgopoulos et al., 1979).

Resistance to EBI's might be based on a multiplicity of different mechanisms, as suggested, for instance, by the existence of at least ten different genes for resistance to imazilil in *A. nidulans* (Van Tuyl, 1977). Resistance to dicarboximides in *Neurospora crassa* is also believed to be multigenic; with other species, however, little is known about the genetic basis of resistance (Beever and Byrde, 1982). Remarkably, resistance to dicarboximides is often accompanied with resistance to aromatic hydrocarbons (Leroux et al., 1977, 1978, 1983; Lyr and Casperson, 1982). In spite of

lack of substantial evidence, it has been suggested that the same chromosomal genes probably control resistance to these various compounds (Leroux et al., 1983). Resistance to both groups of fungicides is often accompanied with increased sensitivity to high osmotic pressure (Beever, 1983).

With a multitude of different genes conferring varying levels of resistance to chemically unrelated fungicides within one fungal species it seems almost impossible to unravel the various resistance mechanisms involved and the genes responsible for each of them. Notwithstanding this obvious lack of knowledge on the genetic basis of resistance, many authors loosely apply the term cross-resistance to describe the simultaneous resistance to various fungicides within one fungal strain. Yet, according to the definition, the term should be used only to indicate resistance to two or more toxicants mediated by the same genetic factor (Georgopoulos, 1977). Alternatively, the term multiple resistance is applied to indicate resistance due to the presence of two dissimilar genes in the same fungal strain each of which confers resistance to one (group of) toxicant(s). In still other instances, the term double resistance is used to refer to resistance 'to more than one unrelated fungicide' (Wild, 1983).

In our experiments, a distinct level of simultaneous resistance to EBI's and dicarboximides (for details, see also below) was only found in two *C. cucumerinum* strains which were both repeatedly UV-irradiated in the selection procedure. However, since the genetic basis of the resistance to EBI's – and *a fortiori* to dicarboximides – in *C. cucumerinum* is unknown (cf. Fuchs et al., 1977), it is impossible to 'classify' this type of resistance as either cross- or multiple resistance. It should be realized that knowledge on the exact type of resistance in such cases is of utmost practical importance: only with cross-resistance between chemically unrelated fungicides, resistance to one group will likely create a hazard to the performance of the other.

The results of the experiments allow of some additional, tentative conclusions. Firstly, both EBI's (De Waard and Van Nistelrooy, 1981) and dicarboximides (this paper) induced [^{14}C]-fenarimol efflux in the wild-type strain of *A. nidulans.* Also natural fungitoxic compounds, like the phytoalexin pisatin, have the same effect (Fuchs et al., 1983). These observations together with comparable earlier ones (De Waard and Van Nistelrooy, 1981) might be indicative of aspecificity of the induction of the efflux mechanism. Secondly, the antagonistic activity of iprodione towards fenarimol, and *vice versa,* in the crossed-paper tests with *A. nidulans,* is suggestive of reciprocal induction of efflux activity by these fungicides. It might mean, that both influence the same cell membrane activity in this species. In *C. cucumerinum*, on the other hand, these two fungicides behaved independently, suggesting absence of a similar inducible efflux mechanism in the latter species. Thirdly, the rather low resistance to iprodione as compared with that to vinclozolin in *C. cucumerinum* might be indicative of differences in resistance mechanism. This somewhat resembles the situation found in *Penicillium expansum* where part of vinclozolin-resistant isolates appeared not to be resistant to iprodione (Rosenberger and Meyer, 1981). Fourthly, the exceptional position of *P. italicum* with respect to natural insensitivity to triadimefon – and triadimenol (results not given) – with normal sensitivity to other EBI's, including other triazoles, suggests involvement of unknown mechanisms of resistance in this species.

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Samenvatting

Gelijktijdige resistentie in schimmels tegen ergosterolbiosyntheseremmers en dicarboximiden

Verscheidene tegen ergosterolbiosyntheseremmers resistente stammen van *Aspergillus nidulans, Cladosporium cucumerinum* en *Penicillium italicum* werden getoetst op resistentie tegen dicarboximiden. Eén der drie onderzochte stammen van *A. nidulans* bezat enige resistentie tegen iprodione en vinchlozoline. Twee van de drie onderzochte stammen van *C. cucumerinum* vertoonden een lage graad van resistentie tegen iprodione en een zeer hoge tegen procymidone en vinchlozoline. Ze waren ook enigermate resistent tegen de fytoalexinen medicarpine en pisatine, maar gevoelig voor het antibioticum pimaricine. Alle onderzochte stammen van *P. italicum* waren voor de drie getoetste dicarboximiden even gevoelig als het wild-type; voorzover onderzocht, bleken deze stammen resistent tegen pimaricine.

Iprodione en, hoewel in mindere mate, vinchlozoline induceerden energie-afhankelijke efflux van fenarimol in *A. nidulans*. In overeenstemming hiermee antagoneerden iprodione en fenarimol elkander in hun activiteit ten opzicht van *A. nidulans*. Ten opzichte van *C. cucumerinum* gedroegen deze fungiciden zich onafhankelijk van elkaar.

De practische consequenties van de waargenomen verschijnselen worden kort aangeduid.

References

Beever, R.E., 1983. Osmotic sensitivity of fungal variants resistant to dicarboximide fungicides. Trans. Br. Mycol. Soc. 80: 327-331.

Beever, R.E. & Byrde, R.J.W., 1982. Resistance to the dicarboximide fungicides. In: J. Dekker & S.G. Georgopoulos (Eds), Fungicide resistance in crop protection. Pudoc, Wageningen, p. 101-117.

Bellincampi, D., Gualandi, G., Monica, E. la, Poley, C. & Morpurgo, G.P., 1980. Membrane-damaging agents cause mitotic non-disjunction in *Aspergillus nidulans*. Mutat. Res. 79: 169-172.

Cooke, B.K., Loeffler, R.S.T. & Pappas, A.C., 1979. The structural rearrangement of iprodione in ethanolic solution. Pestic. Sci. 10: 393-398.

Fuchs, A., Davidse, L.C., Waard, M.A. de & Wit, P.J.G.M. de, 1983. Contemplations and speculations on novel approaches in the control of fungal plant diseases. Pestic. Sci. 14: 272-293.

Fuchs, A., Ruig, S.P. de, Tuyl, J.M. van & Vries, F.W. de, 1977. Resistance to triforine: a nonexistent problem? Neth. J. Pl. Pathol. 83 (Suppl. 1): 189-205.

Fuchs, A. & Viets-Verweij, M., 1975. Permanent and transient resistance to triarimol and triforine in some phytopathogenic fungi. Meded. Fac. Landbouwwet. Rijksuniv. Gent 40: 699-706.

Fuchs, A. & Waard, M.A. de, 1981. Is there cross-resistance between ergosterol biosynthesis inhibitors and dicarboximides? Neth. J. Pl. Pathol. 87: 241 (Abstr.).
Fuchs, A. & Waard, M.A. de, 1982. Resistance to ergosterol-biosynthesis inhibitors. I. Chemistry and phenomenological aspects. In: J. Dekker & S.G. Georgopoulos (Eds), Fungicide resistance in crop protection. Pudoc, Wageningen p. 71-86.
Georgopoulos, S.G., 1977. Development of fungal resistance to fungicides. In: M.R. Siegel & H.D. Sisler (Eds), Antifungal Compounds, Vol. 2. Marcel Dekker, Inc., New York, Basel, p. 439-495.
Georgopoulos, S.G., Sarris, M. & Ziogas, B.N., 1979. Mitotic instability in *Aspergillus nidulans* caused by the fungicides iprodione, procymidone and vinclozolin. Pestic. Sci. 10: 389-392.
Homans, A.L. & Fuchs, A., 1970. Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. J. Chromatogr. 51: 327-329.
Kato, T., Shoami, M. & Kawase, Y., 1980. Comparison of tridemorph with buthiobate in antifungal mode of action. J. Pestic. Sci. 5: 68-79.
Kerkenaar, A., Uchiyama, M. & Versluis, G.G., 1981. Specific effects of tridemorph on sterol biosynthesis in *Ustilago maydis*. Pestic. Biochem. Physiol. 16: 97-104.
Leroux, P., Fritz, R. & Gredt, M., 1977. Etudes en laboratoire de souches de *Botrytis cinerea* Pers., resistantes à la dichlozoline, au dicloran, au quintozene, à la vinchlozoline et au 26019 RP (ou glycophene). Phytopathol. Z. 89: 347-358.
Leroux, P., Fritz, R. & Gredt, M., 1983. Cross-resistance between 3,5-dichloro-phenyl cyclic imide fungicides (dichlozolin, iprodione, procymidone, vinchlozolin) and various aromatic compounds. In: H. Lyr & C. Polter (Eds), Systemische Fungizide und Antifungale Verbindungen. Abh. Akad. Wiss. DDR, 1982, 1N: 79-88.
Leroux, P., Gredt, M. & Fritz, R., 1978. Etudes en laboratoire de souches de quelques phytopathogènes, resistantes à la dichlozoline, à la dicyclidine, à l'iprodione, à la vinclozoline et à divers fongicides aromatiques. Meded. Fac. Landbouwwet. Rijksuniv. Gent 43: 881-889.
Lyr, H. & Casperson, G., 1982. On the mechanism of action and the phenomenon of cross resistance of aromatic hydrocarbon fungicides and dicarboximide fungicides. Acta Phytopathol. Acad. Sci. Hung. 17: 317-326.
Pappas, A.C. & Fisher, D.J., 1979. A comparison of the mechanism of action of vinclozolin, procymidone, iprodione, and prochloraz against *Botrytis cinerea*. Pestic. Sci. 10: 239-246.
Rosenberger, D.A. & Meyer, F.W., 1981. Postharvest fungicides for apples: development of resistance to benomyl, vinclozolin, and iprodione. Plant Dis. 65: 1010-1013.
Tuyl, J.M. van, 1977. Genetics of fungal resistance to systemic fungicides. Thesis Agricultural University, Wageningen.
Waard, M.A. de & Fuchs, A., 1982. Resistance to ergosterol-biosynthesis inhibitors. II. Genetic and physiological aspects. In: J. Dekker & S.G. Georgopoulos (Eds), Fungicide resistance in crop protection. Pudoc, Wageningen, p. 87-100.
Waard, M.A. de & Gieskes, S.A., 1977. Characterization of fenarimol-resistant mutants of *Aspergillus nidulans*. Neth. J. Pl. Pathol. 83 (Suppl. 1): 177-188.
Waard, M.A. de, Groeneweg, H. & Nistelrooy, J.G.M. van, 1982. Laboratory resistance to fungicides which inhibit ergosterol biosynthesis in *Penicillium italicum*. Neth. J. Pl. Pathol. 88: 99-112.
Waard, M.A. de & Nistelrooy, J.G.M. van, 1981. Induction of fenarimol-efflux activity in *Aspergillus nidulans* by fungicides inhibiting sterol biosynthesis. J. Gen. Microbiol. 126: 483-489.
Waard, M.A. de & Nistelrooy, J.G.M. van, 1982. Antagonistic and synergistic activities of various chemicals on the toxicity of fenarimol to *Aspergillus nidulans*. Pestic. Sci. 13: 279-286.
Wild, B.L., 1983. Double resistance by citrus green mould *Penicillium digitatum* to the fungicides guazatine and benomyl. Ann. Appl. Biol. 103: 237-241.