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Laboratory resistance to dicarboximides and ergosterol biosynthesis inhibitors in Penicillium expansum

M.L. GULLINO[1] and M.A. DE WAARD[2]

[1] Istituto di Patologia Vegetale, 10126 Torino, Via Pietro Guiria 15, Torino, Italy
[2] Laboratorium voor Fytopathologie, Binnenhaven 9, 6709 PD Wageningen, the Netherlands

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Dicarboximides and ergosterol biosynthesis inhibitors (EBIs) are two distinct groups of fungicides which are increasingly used for control of *Penicillium expansum,* the pathogen causing blue mold of pome fruits (Burton and Dewey, 1981; Rosenberger, 1981; Tepper, 1982). Resistance to both groups of fungicides is a potential problem (e.g. Dekker and Georgopoulos, 1982). Therefore, it was of interest to study laboratory resistance to these fungicides in this pathogen. The need to do so was also based on reports that iprodione, a dicarboximide, interfered with ergosterol biosynthesis in *Botrytis cinerea* (Pappas and Fisher, 1979). Furthermore, resistance to fungicides of both groups may occasionally be present in the same fungal isolates (Fuchs et al., 1984).

Mass selection of fungicide-resistant isolates of *P. expansum* was carried out on malt agar amended with dicarboximides (iprodione, procymidone, vinclozolin) or EBIs (fenarimol, fenapanil, imazalil, prochloraz) as described earlier for *P. italicum* (De Waard et al., 1982). With all fungicides mentioned resistant isolates were obtained. The degree of resistance of a number of representative isolates to various fungicides was determined (Table 1). Cross-resistance was only obvious to fungicides classified in the same group.

All dicarboximide and EBI-resistant isolates obtained (n = 30) were pathogenic but their virulence ranged from normal (comparable with wild-type) to almost nil. The correct identity of a number of isolates as *P. expansum* was confirmed by the Central Bureau voor Schimmelcultures (Baarn, the Netherlands). Phenotype analysis of mixed-spore inocula of the wild-type and highly virulent resistant isolates during successive generation cycles on apples revealed that in 18 of such pairs tested, the proportion of resistant conidia always decreased from 50% to 10% or less. This indicates that resistant isolates generally have a lower competitive fitness than the wild-type.

The efficacy of various fungicides against *Penicillium* decay incited by dicarboximide or EBI-resistant isolates was tested in dip treatments of apples (Table 2). Under these conditions dicarboximide or EBI resistance was also noticed but again no obvious fungal cross-resistance between the two groups of fungicides was observed.

The results indicate that laboratory resistance to dicarboximides and EBIs in *P. expansum* can easily be selected. However, the relatively low competitive fitness of the resistant isolates tested supports the hypothesis that the risk of development of resistance to these fungicides in practice is relatively low as compared with that to other site-specific fungicides (Dekker, 1982). No apparent fungal cross-resistance bet-

Table 1. Degree of resistance to various dicarboximides and EBIs in isolates of *Penicillium expansum*.

Isolate	Fungicide used for selection	Q-value[1]				
		ipr	vin	fen[2]	ima	eta
IPR 2	iprodione	>30	>30	1	1	1
PRO 1	procymidone	>30	>30	1	3	1
PRO 3	procymidone	>30	>30	1	1	1
PRO 4	procymidone	>30	>30	1	3	1
FEN 14	fenarimol	1	1	>10	100	100
FEN UV5.2	fenarimol	1	1	>10	3	3
FEP 1	fenapanil	1	1	>10	100	33
IMA 3	imazalil	1	1	>10	>100	33

[1] Q-value: ratio between minimal growth inhibitory concentration (MIC) on malt agar for test isolate and wild-type isolate. MIC values of *ipr*odione, *vin*clozolin, *fen*arimol, *ima*zalil and *eta*conazole for the wild-type isolate were 10, 10, 30, 3, and 3 μg ml^{-1}, respectively.

[2] Data from radial growth experiments.

Tabel 2. Efficacy of postharvest treatments with dicarboximides (iprodione, vinclozolin) and EBI fungicides (etaconazole, prochloraz) for control of *Penicillium* decay of apples. Isolates tested were W (wild-type), IPR 2 and PRO 4 (dicarboximide-resistant) and FEN 14 and FEN UV5.2 (EBI-resistant).

Treatment[1]		Diameter of fungal colonies on fruits (% of control treatment[2])				
fungicide	concentration (μg ml^{-1})	W	IPR 2	PRO 4	FEN 14	FEN UV5.2
Iprodione	1000	8	83	66	0	0
	2500	0	95	69	0	0
Vinclozolin	1000	67	83	67	0	0
	2500	5	84	60	0	0
Etaconazole	100	0	7	15	83	100
	250	0	0	0	79	72
Prochloraz	100	0	25	6	44	83
	250	0	0	0	54	110

[1] Fruits were dipped in formulated fungicide solutions for 1 min, inoculated and assessed after 8 days of incubation at 22 °C. Per treatment two apples inoculated at three sites on the equator of the fruits were used.

[2] Diameters in control treatment for isolates W, IPR 2, PRO 4, FEN 14 and FEN UV5.2 were 37, 25, 30, 14 and 12 mm, respectively.

ween the two groups of fungicides was detected. This suggests that an alternating use of compounds of the two groups against *Penicillium* decay can reduce the chance of development of resistance to either one.

Samenvatting

Laboratoriumresistentie tegen dicarboximiden en ergosterol biosynthese remmers in Penicillium expansum

Isolaten van *Penicillium expansum* werden geselecteerd op moutagar met een dicarboximide (iprodion, procymidon, vinchlozolin) of een ergosterol biosynthese remmer (fenarimol, fenapanil, imazalil, prochloraz). De verkregen isolaten vertoonden alleen kruisresistentie tegen fungiciden behorende tot dezelfde groep van middelen. Deze kruisresistentie kon ook in dompelproeven met geïnoculeerde appels worden aangetoond. Alle getoetste isolaten bezaten in mengpopulaties van gevoelige en resistente isolaten op appels een relatief laag competitief vermogen.

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