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On the specificity of the in vitro and in vivo antifungal activity of benomyl

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Abstract

With respect to sensitivity to benomyl in vitro four categories of fungi were distinguished; taxonomically related fungi usually proved to belong to the same category. Within the form-class Deuteromycetes a correlation appeared to exist between sensitivity to this fungicide and morphogenesis of conidia. *Phoma betae*, in contrast to all other *Phoma* spp., was shown to be highly resistant to benomyl. This, however, may be expected on account of its perfect state.

The data on the in vitro antifungal activity of benomyl conformed to those on the effectiveness of benomyl against plant diseases.

Introduction

In the course of a study on the qualitative and quantitative occurrence of soil pathogens in different soil types the use of selective media was considered. Selectivity of a medium depends on the available nutrients, or on the presence of selective specific inhibitors (antibiotics, fungicides, etc.) for which a particular fungus happens to be relatively insensitive. Therefore, a recently available fungicide, benomyl (=1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester), was examined for its selectivity in vitro against fungi, mainly soil fungi. Moreover, the results of experiments in vitro were compared with literature data on the effects of this systemic fungicide in vivo, in order to establish whether the data on the activity in vitro justify a prediction on the effectiveness of benomyl in controlling or preventing plant diseases.

Material and methods

The isolates tested have either been isolated from greenhouse soil (88) or diseased plant material (28) or have been obtained from Centraalbureau voor Schimmelcultures, Baarn (6), Bosbouwproefstation 'De Dorschkamp', Wageningen (3), Plantenziektenkundige Dienst, Wageningen (9), and Instituut voor Plantenziektenkundig Onderzoek, Wageningen (7). All isolates have been tested at 22–25°C on potato dextrose agar (PDA), to which aqueous suspensions of benomyl (50 % wettable powder) were added to give a series of cencentrations required. All concentrations given are based on the commercial preparation used. To arrive at the concentrations of the active ingredient all data should be divided by 2.

Inoculation was carried out by placing 5 mm discs of agar with good growing mycelium upside down on the PDA plates, to which 50 ppm of Vendarcin had been added to suppress bacterial growth. Inhibitory concentrations are expressed as ED_{50}, that is the concentration causing 50 % reduction in radial growth. When the response of a fungal isolate was inconsistent with that of all or most of the other isolates of taxonomically related species, several isolates of the same species were tested, if available.

To determine whether the compound displayed fungicidal or fungistatic activity, in some instances transfers were made from benomyl-containing media onto benomyl-free media.

Results

Results have been summarized in Table 1. From this table it is obvious, that the fungi investigated showed large variations in sensitivity to benomyl. Disregarding some exceptions to be discussed later, the fungi examined could arbitrarily be distinguished in four categories:

a. resistant ($ED_{50} > 100$ ppm): Oomycetes, most of the Zygomycetes, and among the Moniliales (= Hyphomycetes) the Porosporae as well as the two genera of the Annellosporae tested.
b. tolerant (ED_{50} 10–100 ppm): three of the only four Basidiomycetes tested.
c. sensitive (ED_{50} 1–10 ppm): Sphaeropsidales and the Fusaria among the Phialosporae (Moniliales).
d. very sensitive (ED_{50} 0.1–1 ppm): Ascomycetes (see, however, remark in Table 1), and among the Moniliales the Blastosporae, the Phialosporae (with exception of the Fusaria) and the Aleuriosporae.

If only a limited number of representatives of a taxonomic group were tested, no general conclusions as to category of sensitivity have been drawn.

A number of randomly chosen benomyl-treated, sensitive fungi, viz *Aspergillus flavus*, *Aspergillus niger*, *Gilmaniella humicola*, *Penicillium expansum* and *Phoma herbarum*, were subcultured on benomyl-free media. All these proved to be viable; in the case of *Gilmaniella humicola* sub-culturing was successful even after contact with up to 500 ppm benomyl for 4 weeks, although in this and other instances a second transfer was needed to dilute the benomyl present to such a low concentration as to enable growth. Thus, benomyl seems to exert a fungistatic rather than a fungicidal action (cf. Hine et al., 1969; Al-Beldawi and Pinckard, 1970).

Discussion

The conclusion might be drawn that the response of fungi towards benomyl is highly predictable on the basis of their taxonomic position (Table 1). No activity can be expected towards Oomycetes and Zygomycetes, and among the Moniliales towards the fungi producing porospores or annellospores[1]. The species among the Moniliales with blasto-, phialo- and aleuriosporic spore formation, however, appear to be highly sensitive to this fungicide. Thus, a correlation seems to exist between response towards benomyl and sporogenesis. Also in the other groups most fungi tested appeared to be

[1] However, this seems not to be true for all annellosporic species, as is shown by Venturia inaequalis (see Table 2), the imperfect state of which is characterized by annellosporic spore formation.

sensitive to highly sensitive to benomyl. Literature data on in vitro fungitoxicity of benomyl (e.g. Ogawa et al., 1968; Bompeix and Morgat, 1969; Davis and Pinckard, 1969; Hine et al., 1969; Al-Beldawi and Pinckard, 1970; Edgington and Khew, 1970) corroborate our hypothesis: without exception all fungi mentioned there belong to the same category as related fungi examined by us.

Within the Sphaeropsidales all four isolates of *Phoma betae* examined by us proved to be very resistant to benomyl[1], contrary to all other isolates of this form-group tested. However, according to its rarely occurring perfect state, viz *Pleospora bjoerlingii* (cf. Björling, 1944; Byford, 1963), *Phoma betae* can be considered to be closely related to the porosporic and 'thus' insensitive form-species *Stemphylium*, the perfect state of

[1] Also Ogawa et al. (1968) found the latter species to be insensitive for benomyl.

Table 1. In vitro fungistatic spectrum of benomyl. (Fungistatic activity expressed as ED_{50}, based on 50 % WP.)

Fungal species tested*	I/R**	ED_{50} (in ppm)	Category of sensitivity
OOMYCETES			
*Phytophthora cryptogea****	1/3	100–500	a
Pythium irregulare	1/3	> 500	a
Pythium ultimum	1/1	100–500	a
ZYGOMYCETES			
Circinella minor	1/2	ca 200	a
Circinella spinosa	1/1	200–500	a
Mortierella alpina	2/3	ca 10	b
Mortierella candelabrum	2/4	10–25	b
Mortierella elongata	3/5	ca 25	b
Mortierella isabellina	1/2	> 500	a
Mortierella vinacea	1/2	> 500	a
Mucor circinelloïdes	1/2	> 500	a
Mucor hiemalis	1/2	100–500	a
Mucor racemosus	1/2	> 500	a
Phycomyces blakesleanus	1/2	100–500	a
Zygorhynchus moelleri	2/3	ca 500	a
ASCOMYCETES Most of the Ascomycetes are classified according to their conidial state. See: Deuteromycetes.			
Chaetomium globosum	1/2	0.1–1	d
Coniochaeta cf. *xylariispora*	1/1	0.1–1	d
Gaeumannomyces graminis, syn. *Ophiobolus graminis*	1/1	0.1–1	d
Leptosphaerulina australis	1/1	1–5	c
Pseudeurotium ovale	1/1	0.1–1	d
Pseudeurotium zonatum	1/1	< 0.1	d

Table 1 (Continued).

Fungal species tested*	I/R**	ED_{50} (in ppm)	Category of sensitivity
DEUTEROMYCETES			
Sphaeropsidales			
Ascochyta chrysanthemi (*Mycosphaerella ligulicola*)****	1/5	1–5	c
Ascochyta lycopersici (*Didymella lycopersici*)	1/5	1–5	c
Ascochyta pinodes (*Didymella pinodes*)	1/1	5–10	c
Ascochyta pisi	2/2	1–5	c
Brunchorstia pinea (*Scleroderris lagerbergii*)	1/1	0.1–1	d
Coniothyrium fuckelii (*Leptosphaeria coniothyrium*)	2/4	1–5	c
Coniothyrium minitans	1/1	0.1–1	d
Coniothyrium sp.	1/1	ca 5	c
Darluca filum	1/2	ca 5	c
Phoma betae (*Pleospora bjoerlingii*)	4/7	ca 500	a
Phoma chrysanthemicola	1/1	1–5	c
Phoma doliolum (*Leptosphaeria doliolum*)	1/1	1–5	c
Phoma eupyrena	2/2	1–5	c
Phoma exigua	1/1	1–5	c
Phoma fimeti	2/2	1–5	c
Phoma glomerata	1/1	1–5	c
Phoma herbarum	2/2	1–5	c
Phoma medicaginis	1/1	1–5	c
Phoma prunicola	1/1	1–5	c
Phoma sp.	1/1	5–10	c
Phomopsis sclerotioides	1/2	ca 1	c
Moniliales			
Blastosporae			
Aureobasidium pullulans	1/1	0.1–1	d
Botrytis cinerea (*Sclerotinia fuckeliana*)	1/2	1–5	c
Chromelosporium sp. (*Peziza ostracoderma*)	2/2	1–5	c
Cladosporium herbarum (*Mycosphaerella tassiana*)	1/2	0.1–1	d
Monilia sitophila (*Neurospora sitophila*)	1/1	0.1–1	d
Periconia igniaria	1/1	0.1–1	d
Porosporae			
Alternaria tenuis	1/1	ca 200	a
Alternaria phragmospora	1/1	ca 500	a
Curvularia trifolii	1/3	ca 50	b
Dendryphion penicillatum	1/1	ca 500	a
Drechslera avenae (*Pyrenophora avenae*)	4/6	50–100	b

Table 1 (Continued).

Fungal species tested*	I/R**	ED_{50} (in ppm)	Category of sensitivity
Drechslera sp.	1/1	ca 100	a
Helminthosporium oryzae (*Cochliobolus miyabeanus*)	2/1	10–100	b
Helminthosporium sativum (*Cochliobolus sativus*)	2/3	100–500	a
Stemphylium botryosum (*Pleospora herbarum*)	1/2	ca 500	a
Ulocladium atrum	1/1	> 500	a
Arthrosporae			
Chrysosporium pannorum	2/2	0.1–1	d
Geotrichum sp.	1/1	> 500	a
Oidiodendron griseum	1/1	0.1–1	d
Phialosporae			
Acremonium ochraceum	1/1	1–5	c
Acremonium strictum, syn. *Cephalosporium acremonium* auct.	1/3	1–5	c
Aspergillus amstelodami (*Eurotium amstelodami*)	1/1	0.1–1	d
Aspergillus flavus	1/2	0.1–1	d
Aspergillus fumigatus	1/2	0.1–1	d
Aspergillus niger	1/2	0.1–1	d
Aspergillus ochraceus	1/1	0.1–1	d
Cladorrhinum foecundissimum	1/1	0.1–1	d
Cylindrocarpon destructans (*Nectria radicicola*)	1/2	1–5	c
Fusarium avenaceum (*Gibberella avenacea*)	1/2	1–10	c
Fusarium oxysporum	2/3	1–10	c
Fusarium oxysporum f. *pisi*	1/2	1–10	c
Fusarium redolens	1/2	1–10	c
Fusarium solani (*Nectria haematococca*)	1/2	1–50	b, c
Fusarium solani f. *cucurbitae* (*Nectria haematococca* f. *cucurbitae*)	1/1	1–10	c
Fusarium tabacinum (*Plectosphaerella cucumeris*)	1/2	0.1–1	d
Gliocladium roseum	1/1	0.1–1	d
Myrothecium verrucaria	1/2	0.1–1	d
Penicillium brefeldianum (*Eupenicillium brefeldianum*)	1/1	0.1–1	d
Penicillium brevicompactum	1/1	0.1–1	d
Penicillium expansum	1/2	0.1–1	d
Penicillium helicum (*Talaromyces helicus*)	1/1	0.1–1	d
Penicillium notatum	1/1	0.1–1	d
Penicillium vermiculatum (*Talaromyces vermiculatus*)	1/1	0.1–1	d
Penicillium wortmannii (*Talaromyces wortmannii*)	1/1	0.1–1	d
Phialophora cinerescens	1/3	0.1–1	d
Phialophora hoffmannii group	1/1	1–5	c

Table 1 (Continued).

Fungal species tested*	I/R**	ED_{50} (in ppm)	Category of sensitivity
Phialophora malorum	1/1	<0.1	d
Phialophora sp.	1/1	0.1–1	d
Stachybotrys chartarum, syn. *S. atra*	1/1	1–5	c
Thielaviopsis basicola	1/4	1–5	c
Trichoderma viride (*Hypocrea rufa*)	2/4	0.1–1	d
Verticillium albo-atrum	1/1	0.1–1	d
Verticillium dahliae	2/3	ca 1	c
Verticillium lecanii	1/1	1–5	c
Aleuriosporae			
Botryotrichum piluliferum (*Chaetomium piluliferum*)	1/1	0.1–1	d
Gilmaniella humicola	2/3	0.1–1	d
Humicola grisea	1/1	0.1–1	d
Trichocladium asperum	1/1	0.1–1	d
Trichocladium opacum	1/3	0.1–1	d
Trichophyton terrestre	1/2	0.1–1	d
Annellosporae			
Doratomyces microsporus	2/2	ca 500	a
Doratomyces purpureofuscus	1/1	200–500	a
Doratomyces stemonitis	2/2	200–500	a
Scopulariopsis acremonium	1/1	> 500	a
Scopulariopsis brevicaulis	1/1	> 500	a
Scopulariopsis paisii (*Microascus desmosporus*)	1/1	> 500	a
Radulasporae = Sympodulosporae			
Tritirachium album	1/2	1–5	c
Rhinocladiella mansonii	2/2	< 0.1	d
MYCELIA STERILIA			
Rhizoctonia solani	1/3	1–5	c
BASIDIOMYCETES			
Aphyllophorales			
Fomes annosus	1/2	ca 10	b
Peniophora gigantea	1/2	10–25	b
Polyporus adustus	1/2	10–25	b
Polyporus betulinus	1/2	100–200	a

* Classified according to J. A. von Arx, 'Pilzkunde' (1967), and G. L. Barron, 'The genera of Hyphomycetes from soil' (1968).
** I/R Number of isolates/number of replicates.
*** Tested on PDA without Vendarcin.
**** Names between brackets refer to perfect state.

Tabel 1. Het fungistatisch spectrum van benomyl op grond van proeven in vitro. (De fungistatische activiteit is uitgedrukt als ED_{50} en gebaseerd op de formulering 50 %WP)

Table 2. Effectiveness of benomyl against plant diseases as derived from literature data; – no effect, + considerable to complete protection.

Fungal species tested*	– or +	Host plant	Reference
CHYTRIDIOMYCETES			
Olpidium brassicae	–	*Solanum villosum*	personal observation
OOMYCETES			
Bremia lactucae	–	lettuce	Waffelaert (1969)
Phytophthora spp.	–	–	Delp and Klopping (1968b)
Plasmopara viticola	–	grape vine	Delp and Klopping (1968b)
Pythium spp.	–	–	Delp and Klopping (1968b)
ZYGOMYCETES			
Rhizopus stolonifer	–	peach	Ogawa et al. (1968)
Rhizopus spp.	–	–	Delp and Klopping (1968b)
ASCOMYCETES			
Botryotinia squamosa	+	onion	Lafon and Bugaret (1969)
Ceratocystis ulmi	+	elm	Biehn and Dimond (1970a)
Claviceps purpurea	+	*Poa pratensis*	Hardison (1968)
Coccomyces hiemalis	+	cherry	Delp and Klopping (1968a, b), Cimanowski et al. (1970)
Diplocarpon rosae	+	rose	Delp and Klopping (1968a, b), Engelhard (1969)
Erysiphe cichoracearum	+	cantaloup	McKittrick and Troutman (1969)
		cucumber	Hammett (1968), Cole et al. (1970a)
		melon	Lafon and Bugaret (1969)
		pumpkin	Hammett (1968)
		squash	Delp and Klopping (1968a, b)
Erysiphe graminis	+	*Poa pratensis*	Hardison (1968)
Erysiphe graminis f. sp. *tritici*	+	wheat	Hammett (1968), Johnston (1970)
Erysiphe polygoni	+	bean	Chancogne and Gredt (1969)
		cucumber	Chancogne and Gredt (1969)
		sweet pea	Hammett (1968)
Eutypa armeniacae	+	apricot	Moller and Carter (1969)
Gloeotinia temulenta	+	*Lolium perenne*	Hardison (1968)
Guignardia bidwellii	+	grape vine	Delp and Klopping (1968a, b)
Microthyriella rubi	+	apple	Delp and Klopping (1968a, b)
Monilinia fructicola	+	cherry	Ogawa et al. (1968)
		nectarine	Ogawa et al. (1968)
		peach	Chandler (1968), Delp and Klopping (1968a, b), Ogawa et al. (1968)
Monilinia laxa	+	cherry	Ogawa et al. (1968)
Mycosphaerella musicola	+	banana	Stover (1969)
Nectria galligena	+	apple	McDonnell (1970)
Physalospora obtusa	+	apple	Delp and Klopping (1968a, b)
Podosphaera leucotricha	+	apple	Delp and Klopping (1968a, b), Hammett (1968), Gilpatrick (1969), Cimanowski et al. (1970)
Podosphaera oxyacanthae	+	cherry	Gilpatrick (1969)

Table 2 (Continued).

Fungal species tested*	– or +	Host plant	Reference
Sclerotinia homoeocarpa	+	*Agrostis palustris*	Cole et al. (1968)
		Agrostis tenuis	Cole et al. (1968), Massie et al. (1968)
Sphaerotheca fuliginea	+	cantaloup	Paulus et al. (1969)
		cucumber	Schroeder and Provvidenti (1968), Paulus et al. (1969)
		squash	Schroeder and Provvidenti (1968)
Sphaerotheca macularis	+	raspberry	Freeman and Pepin (1967)
		strawberry	Freeman and Pepin (1967)
Sphaerotheca pannosa var. rosae	+	rose	Delp and Klopping (1968a, b)
Venturia inaequalis	+	apple	Connor and Heuberger (1968), Delp and Klopping (1968a, b), Gilpatrick (1969), McIntosh (1969), Miller (1970)
DEUTEROMYCETES			
Sphaeropsidales			
Diplodia natalensis	+	orange	Brown (1968)
		tangerine	Brown (1968)
Gloeodes pomigena	+	apple	Delp and Klopping (1968a, b)
Phomopsis citri	+	orange	Brown (1968)
		tangerine	Brown (1968)
Phomopsis sclerotioides	+	cucumber	Crüger (1969)
Septoria apii-graveolentis	+	celery	Delp and Klopping (1968a, b)
Septoria macropoda var. *septulata*	+	*Poa pratensis*	Hardison (1968)
Trichoseptoria fructigena	+	apple	Bompeix and Morgat (1969)
Melanconiales			
Colletotrichum gloeosporioides	+	tangerine	Brown (1968)
Colletotrichum lindemuthianum	+	bean	Delp and Klopping (1968a, b)
Colletotrichum orbiculare	+	cucumber	Doma (personal communication)
Colletotrichum phomoides	+	tomato	Delp and Klopping (1968b)
Gloeosporium musarum	+	banana	Long (1970)
Gloeosporium perennans	+	apple	Bompeix and Morgat (1969)
Melanconium fuligineum	+	grape vine	Delp and Klopping (1968a, b)
Sphaceloma fawcetti	+	mandarin	Hearn and Childs (1969)
Moniliales			
Blastosporae			
Botrytis cinerea	+	blueberry	Delp and Klopping (1968a, b)
		geranium	Manning and Glickman (1969)
		grape vine	Chancogne and Gredt (1969)
		raspberry	Freeman and Pepin (1967)
		strawberry	Freeman and Pepin (1967)
Oidium sp.	+	tamarillo	Hammett (1968)
Porosporae			
Alternaria spp.	–	–	Delp and Klopping (1968a, b)
Curvularia trifolii f. sp. *gladioli*	+	gladiolus	Forsberg (1969)

Table 2 (Continued).

Fungal species tested*	– or +	Host plant	Reference
Helminthosporium sorokianum	–	wheat	Johnston (1970)
Helminthosporium vagans	–	*Poa pratensis*	Jackson (1970)
Helminthosporium spp.	–	–	Delp and Klopping (1968a, b)
Arthrosporae			
Geotrichum candidum	–	tangerine	Brown (1968)
Phialosporae			
Cylindrocladium floridanum	+	azalea	Horst and Hoitink (1968)
		lilac	Horst and Hoitink (1968)
		rhododendron	Horst and Hoitink (1968)
		sweet-william	Horst and Hoitink (1968)
Cylindrocladium parvum	+	lilac	Horst and Hoitink (1968)
		rhododendron	Horst and Hoitink (1968)
Cylindrocladium scoparium	+	azalea	Horst and Hoitink (1968)
Fusarium oxysporum f. *gladioli*	+	gladiolus	Forsberg (1969)
		iris	Gould and Miller (1970)
Fusarium oxysporum f. *lycopersici*	+	tomato	Biehn and Dimond (1969, 1970b), Fuchs et al. (1970)
Fusarium oxysporum f. *pisi*	+	pea	Fuchs et al. (1970)
Fusarium spp.	+	cotton	Delp and Klopping (1968a, b)
Penicillium digitatum	+	lemon	Gutter (1969b)
		mandarin	Harding (1968)
		orange	Brown (1968), Delp and Klopping (1968b), Harding (1968), Gutter (1969a)
		tangelo	Harding (1968)
		tangerine	Brown (1968), Harding (1968)
		tangor	Harding (1968)
Penicillium expansum	+	apple	Spalding et al. (1969)
Penicillium italicum	+	mandarin	Harding (1968)
		orange	Harding (1968)
		tangelo	Harding (1968)
		tangerine	Harding (1968)
		tangor	Harding (1968)
Piricularia oryzae	+	rice	Delp and Klopping (1968a, b)
Thielaviopsis basicola	+	bean	Papavizas et al. (1970)
		carrot	Reynolds (1970)
		Citrus excelsa	Allen and Hine (1969)
		tobacco	Saenger (1970)
Verticillium albo-atrum	+	cotton	Delp and Klopping (1968a, b), Erwin et al. (1968), Rawlins and Booth (1968), Erwin (1969)
		potato	Biehn (1969, 1970a, b)
Aleuriosporae			
Clasterosporium carpophilum	+	peach	Delp and Klopping (1968 a, b)
Cercospora apii	+	celery	Delp and Klopping (1968a, b)
Cercospora beticola	+	sugar-beet	Delp and Klopping (1968a, b)
Fusicladium effusum	+	pecan	Worley and Harmon (1968)

Table 1 (Continued).

Fungal species tested*	— or +	Host plant	Reference
MYCELIA STERILIA			
Rhizoctonia solani	+	bean	Papavizas et al. (1970)
		cotton	Al-Beldawi and Pinckard (1968, 1970), Delp and Klopping (1968a, b)
		potato	Biehn (1969)
Sclerotium cepivorum	+	garlic	Lafon and Bugaret (1969)
Sclerotium rolfsii	—	carrot	Reynolds (1970)
		—	Delp and Klopping (1968a, b)
BASIDIOMYCETES			
Gymnosporangium juniperi-virginianae	—	apple	Delp and Klopping (1968b), Gilpatrick (1969)
Puccinia striiformis	+	*Poa pratensis*	Hardison (1968)
Urocystis agropyri	+	*Poa pratensis*	Hardison (1968)
		wheat	Metcalfe and Brown (1969)
Uromyces phaseoli var. *typica*	+	bean	Delp and Klopping (1968a, b)
Ustilago bullata	+	*Bromus willdenowii*	Latch (1969)
Ustilago nuda	+	wheat	Metcalfe and Brown (1969)
Ustilago striiformis	+	*Agrostis palustris*	Hardison (1968)
		Poa pratensis	Halisky et al. (1968, 1969), Hardison (1968), Cole et al. (1970b), Jackson (1970)
Ustilago tritici	+	wheat	Tyler (1969), Johnston (1970)

* Names of fungal species according to the authors cited.

Tabel 2. De werking van benomyl tegen planteziekten, ontleend aan literatuurgegevens; — geen effect, + goede tot volledige bescherming.

which also belongs to the genus *Pleospora*, viz *P. herbarum*. Therefore, the resistance of *Phoma betae* against benomyl is plausible considering its perfect state. It is notable though, that most of the resistant species of the Deuteromycetes having an ascigerous state belong to the family of the *Pleosporaceae*, viz to the genera *Pleospora*, *Pyrenophora*, and *Cochliobolus*. According to Von Arx (1967) this family also includes the genera *Leptosphaeria* and *Leptosphaerulina*, the species of which, however, proved to be sensitive to the fungicide.

Within one genus in some instances a rather large variation in sensitivity towards benomyl is shown. For instance, among the genus *Mortierella* most species tested are relatively tolerant (*M. alpina*, *M. candelabrum* and *M. elongata*), whereas representatives of the *Isabellina*-group (*M. isabellina*, *M. vinacea*) are very resistant. Variations within groups of which only a small number of species were examined such as the Basidiomycetes should not be given too much attention.

The specificity of the action of benomyl is used in this laboratory in the purification of cultures of tolerant fungal species from occasional contaminants, e.g. *Penicillia*, which are sensitive to this compound. Moreover, the fungicide can be expected to be very useful in the isolation of benomyl-tolerant fungi by adding it to the selective media employed. Unfortunately, the ubiquity of *Mucor* and other Zygomycetes in soil renders benomyl less suitable for the selective isolation of soil pathogens.

Since distinct differences in sensitivity to benomyl had been found in experiments in vitro, it seemed of interest to compare our data with those on the effectiveness of benomyl in controlling or preventing plant diseases, as derived from literature. This comparison revealed an almost perfect correlation (Table 2). It suggests that benomyl (or its conversion product benzimidazole carbamic acid, methyl ester (cf. Clemons and Sisler, 1969; Peterson and Edgington, 1969, 1970; Fuchs et al., 1970)) displays its fungistatic activity without the mediation of plant metabolism. This implies that the activity of benomyl in vivo can be predicted with great certainty from data on in vitro inhibition of fungal growth (see, however, note on p. 300). Only a single apparent exception to the correlation mentioned should be noted: according to Forsberg (1969) this fungicide 'appears to be a promising material for control of *Curvularia* rots of gladiolus', although species belonging to the genus *Curvularia* may be expected to be tolerant to benomyl. The differential effect of benomyl against diseases caused by *Sclerotium* spp., on the other hand, can be readily explained by the fact that the representatives within this form-genus might not at all be related.

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Note

After completion of the manuscript, the authors received a photocopy of a manuscript by L. V. Edgington, K. L. Khew and G. L. Barron, entitled 'Fungitoxic spectrum of benzimidazole compounds', which has been submitted to Phytopathology. In their publication, the authors arrived at almost the same conclusions as drawn in the present article.

Samenvatting

Over de specificiteit van de fungitoxische activiteit van benomyl in vitro en in vivo
Ten aanzien van de gevoeligheid voor het systemische fungicide benomyl werden vier categorieën van schimmels onderscheiden; taxonomisch verwante schimmels bleken gewoonlijk tot dezelfde categorie te behoren. Binnen de vormklasse der Deuteromycetes bleek een correlatie te bestaan tussen de gevoeligheid voor benomyl en de morfogenese der conidiën. In tegenstelling tot alle andere onderzochte *Phoma* soorten bleek *Phoma betae* in hoge mate resistent tegen benomyl; dit is echter, gelet op het perfecte stadium, toch aannemelijk.

De resultaten ten aanzien van de activiteit van benomyl in vitro bleken in overeenstemming met die betreffende de werking van dit fungicide in vivo.

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