Fungitoxic Spectrum of Benzimidazole Compounds

L. V. Edgington, K. L. Khew, and G. L. Barron

Professor, Graduate student, and Professor, respectively, Department of Botany, University of Guelph, Guelph, Ontario, Canada.

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ABSTRACT

Fungitoxicity of three benzimidazole compounds was examined in vitro. Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] was extremely toxic to a wide spectrum of fungi, but was nontoxic to certain taxonomic groups. Among the Deuteromycetes, the sensitive groups were Phialosporae, Arthrosporae, Sympodulosporae, and Aleuriosporae, while only some genera of Annellosporae and Blastosporae were sensitive; all Porosporae were insensitive. Within the Blastosporae, only those genera having perfect stages in the Sclerotiniaceae of the Euascomycetes were sensitive to benomyl, while the yeast, *Candida,* with a perfect stage in the Hemiascomycetes was resistant. Within the Porosporae, benomyl was nontoxic to such important pathogens as *Alternaria, Drechslera, Bipolaris, Stemphylium,* and *Curvularia.* A comparison of benomyl with thiabendazole and Bay 33172 [2-(2-furyl)-benzimidazole] revealed an identical pattern of selective fungitoxicity. Consequently, the selective toxicity of all three compounds depends on the benzimidazole portion of the molecule. Phytopathology 61:42-44.

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Three benzimidazole compounds have been reported to control numerous plant diseases and to be systemic in plants. If we compare the chemical structure of these three fungicides (Fig. 1), the similarity is apparent, especially when we consider that benomyl loses the butylcarbamoyl in aq media as reported by Clemons & Sisler (3). Activity of a benzimidazole fungicide, thiabendazole, against plant pathogens was first reported by Staron & Allard (8), who found lack of toxicity to the Phycomycetes, *Pythium* and *Phytophthora,* and also to the Deuteromycete, *Phoma betae,* as well as bacteria, yeasts, and Actinomycetes. They demonstrated that thiabendazole was toxic to numerous fungi, but failed to consider taxonomic relationships. In 1967, Schuhman (6) noted the similarity in diseases controlled by seed treatment with furidazol [Bay 33172, 2-(2 furyl)-benzimidazole] and thiabendazole. A third benzimidazole compound, benomyl, was found by Delp & Klopping (5) to affect a wide range of fungi, but poor control was obtained for diseases caused by species of Phycomycetes and by *Alternaria, Helminthosporium, Sclerotium rolfsii,* and *Gymnosporangium juniperi-virginianae.* With the exception of Delp & Kloppings' reference to Phycomycetes, no attempt has been made to relate toxicity of these compounds to distinct taxonomic groups of fungi. We evaluated benomyl against a wide range of fungi, then compared the spectrum of toxicity of benomyl with the other two benzimidazole compounds.

MATERIALS AND METHODS.—The fungicides were dissolved in acetone to give stock solutions, and 1 ml of each was dispensed into 100 ml of potato-dextrose agar (PDA) at 50 C. Discs of fungal mycelium growing on PDA were placed in the center of petri plates containing 100, 50, 20, 10, and 1 ppm of the fungicide in PDA. Growth was measured and compared with untreated checks to calculate percentage inhibition of growth. When the ED_{50} (concn inhibiting growth by 50%) was less than 1 ppm, a further test was made using 7, 5, 3, 0.5, 0.1, 0.05, and 0.01 ppm. Tests using Basidiomycetes were made on malt extract agar for better growth. Three replicates were used for each fungus at each of the above concn.

The percentage of growth inhibition was plotted on a probability scale against the log of the concn of the fungicide, and the ED_{50} determined. Fungi which have an ED_{50} of 50 ppm or greater were considered insensitive to the fungicide, those with an ED_{50} of between 1 and 10 ppm as moderately sensitive, and those with an ED_{50} of less than 1 ppm as highly sensitive.

Fungi in the Deuteromycetes were classified according to the system proposed by Barron (1), while those in the Basidiomycetes were classified according to Singer (7).

RESULTS AND DISCUSSION.—An initial experiment (Table 1) indicated that certain groups of Deuteromycetes were highly sensitive; viz: Phialosporae, Arthrosporae, Sympodulosporae, and Aleuriosporae. Within the Blastosporae there appeared to be inconsistencies. *Cladosporium fulvum, Botrytis sp.,* and *Monilia cinerea* were highly sensitive. The latter two have sexual states in the Sclerotiniaceae of the Euascomycetes. *Candida humicola,* which has a sexual stage in the Hemiascomycetes, was insensitive to benomyl. The spectrum of toxicity for this taxonomic group should probably be considered in relation to the perfect state.

With the single exception of *Torula herbarum,* all members of the Porosporae were insensitive. Barron (1), however, draws attention to the atypical method of porospore production in *Torula,* and it is possible that the genus is phylogenetically distinct from the remainder of the group.

Within the Annellosporae, *Leptographium* sp. and *Pestalotia* sp. were highly sensitive while *Scopulariopsis brevicaulus* was insensitive. *Leptographium* and *Graphium* are related through the sexual state, *Ceratocystis.*

TABLE 1. Toxicity of benomyl to fungi in potato-dextrose agar[a]

Fungi	ED_{50} (ppm)[b]
DEUTEROMYCETES	
Phialosporae:	
Verticillium albo-atrum Reinke & Berth.	0.2 ± 0.05
Penicillium gladioli McCull. & Thom	0.2 ± 0.05
Aspergillus niger v. Tiegh.	4.0 ± 0.5
Colletotrichum lagenarium (Pass.) Ell. & Halst.	0.2 ± 0.05
Fusarium oxysporum f. *lycopersici* (Sacc.) Snyd. & Hans.	0.8 ± 0.05
Thielaviopsis basicola (Berk. & Br.) Ferr.	0.2 ± 0.05
Porosporae:	
Alternaria solani Sor.	>100
Bipolaris sorokinianum (Sacc.) Shoemaker	>100
Dichotomophthora indica Rao	>100
Stemphylium botryosum Wallr.	>100
Drechslera sp. (ex Portulaca)	>100
Curvularia geniculata (Tracy & Earle) Boedijn	> 50
Torula herbarum Link ex Fr.	0.3 ± 0.05
Blastosporae:	
Cladosporium fulvum Cooke	0.3 ± 0.05
Botrytis sp.	0.4 ± 0.05
Monilia cinerea f. americana Wormold	0.5 ± 0.04
Candida humicola (Daszewska) Diddens & Lodder	>100
Arthrosporae:	
Geotrichum sp.	0.13 ± 0.003
Amblyosporium botrytis Fres.	0.04 ± 0.005
Chrysosporium sp.	0.5 ± 0.12
Oidiodendron truncatum Barron	0.6 ± 0.02
Annellosporae:	
Leptographium sp.	0.2 ± 0.05
Pestalotia sp.	0.08 ± 0.005
Scopulariopsis brevicaulis (Sacc.) Bain.	>100
Sympodulosporae:	
Graphium ulmi Schwarz	0.2 ± 0.05
Geniculisporium serpens Chesters & Greenhalgh	< 1
Nodulisporium sp.	0.08 ± 0.005
Aleuriosporae:	
Humicola sp.	0.06 ± 0.005
Pithomyces chartarum (Berk. & Curtis) M. B. Ellis	0.1 ± 0.05
Keratinomyces ajelloi Vanbreus.	0.12 ± 0.004
PHYCOMYCETES	
Mucor sp.	>100
Rhizopus nigricans Ehr.	>100
Cunninghamella echinulata Traxt.	>100
Pythium ultimum Trow	>100
Thamnidium elegans Link ex Fres.	100
BASIDIOMYCETES	
Thanatephorus cucumeris (Frank) Donk	3.0 ± 0.5
Polyporus giganteus Pers. ex. Fr.	6.0 ± 0.5
Ustilago maydis (DC.) Corda	1.4 ± 0.2
Coprinus sp.	100

[a] Growth of mycelium in media containing benomyl was compared with growth in untreated media.

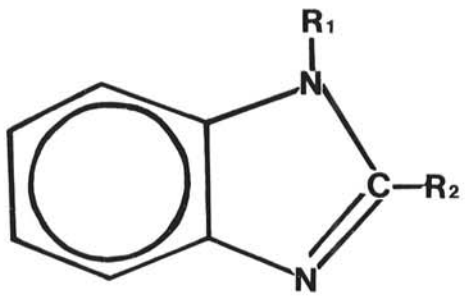
[b] Concentration which inhibits growth by 50% plus or minus the variation among three replicates.

TABLE 2. ED_{50} of benomyl to Basidiomycetes in malt agar[a]

Fungi	ED_{50} (ppm)[b]
HETEROBASIDIAE	
I AURICULARIALES	
1. *Auricularia auricularis* (Gray) Martin	< 2
EUBASIDIAE	
II POLYPORALES	
(Aphyllophorales)	
THELEPHORACEAE	
1. *Corticium vellereum* Ellis & Cragin	>125
2. *Corticium galactinum* (Fr.) Burt	< 31
3. *Hymenochaete tabacina* Sow. ex Lév.	>125
4. *Peniophora gigantea* (Fr.) Massee	31
5. *Trechispora raduloides* (Karst.) Rogers	< 31
HYDNACEAE	
1. *Hericium erinaceum* (Bull. ex Fr.) Pers.	< 2
2. *Odontia bicolor* (Alb. & Schw. ex. Fr.) Quél.	< 31
MERULIACEAE	
1. *Merulius himantioides* Fr.	>125
2. *Merulius tremellosus* Schrad. ex. Fr.	>125
POLYPORACEAE	
1. *Fomes annosus* (Fr.) Cke.	< 8
2. *Fomes igniarius* (L. ex Fr.) Gill.	< 8
3. *Polyporus hirsutus* Wulf. ex Fr.	< 8
4. *Polyporus sulphureus* Bull. ex Fr.	> 31
5. *Poria subacida* (Pk.) Sacc.	< 8
6. *Trametes serialis* Fr.	< 31
III AGARICALES	
POLYPORACEAE	
1. *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer	< 31
2. *Lentinus lepidens* Fr.	<125
3. *Schizophyllum commune* Fr.	< 31
TRICHOLOMETACEAE	
1. *Lyophyllum ulmarium* (Bull. ex Fr.) Kühner	8
2. *Flammulina velutipes* (Curt ex Fr.) Sing.	> 31
AMANITACEAE	
1. *Amanita muscaria* (L. ex Fr.) Hooker	< 2
AGARICACEAE	
1. *Armillaria mellea* (Vahl ex Fr.) Kumm.	8
2. *Agaricus bisporus* (Lange) Imbach	< 31
COPRINACEAE	
1. *Coprinus lagopus* Fr.	< 31
STROPHARIACEAE	
1. *Naematoloma sublateritium* (Fr.) Karst.	< 31
2. *Pholiota adiposa* (Fr.) Kumm.	< 2
BOLETECEAE	
1. *Boletus elegans* Schum.	>125

[a] Growth of mycelium in media containing 3, 8, 31, and 125 ppm benomyl was compared with growth in untreated media.

[b] Concn which inhibited growth by 50%.



Benzimidazole

Common Name	Substituents R_1	R_2
Benomyl	Butylcarbamoyl	Methylcarbamate
Thiabendazole	H	4-Thiazolyl
Furidazol	H	2-Furyl

Fig. 1. Chemical structure of three benzimidazole fungicides.

Graphium ulmi, in the Sympodulosporae, was highly sensitive to benomyl. We have no explanation for the large difference in toxicity of benomyl to the other two genera of Annellosporae, especially since *S. brevicaulis* has a perfect state in the Microascales which places it relatively close to *Ceratocystis.*

Among the Phycomycetes the lack of toxicity is in agreement with previous reports (5).

The four Basidiomycetes initially tested varied from moderately sensitive to insensitive. Consequently, a larger selection was investigated (Table 2). The results failed to show any clear taxonomic response to benomyl. These experiments were repeated and the same results were obtained. Generally, Basidiomycetes varied from moderately sensitive to insensitive. *Ustilago maydis* in the Ustilaginaceae, however, was almost highly sensitive (ED_{50} of 1.4 ppm) (Table 1), and benomyl has generally been reported effective for several smut diseases. Perhaps a definite taxonomic specificity exists for the Ustilaginaceae, an important group of plant pathogens. Cowling (4) also failed to show a response of Basidiomycetes to 10 wood preservative chemicals along taxonomic lines.

When a comparison was made of the spectrum of fungi sensitive to three benzimidazole compounds, the similarity was very obvious. Since the butylcarbamoyl moiety of benomyl is rapidly hydrolyzed in media (3) (such as agar), the similarity of the three compounds is even more striking than it initially appears to be. This leads us to postulate that the specificity of the three compounds for certain taxonomic groups is determined by the benzimidazole moiety and not by the R_2 group. However, benzimidazole alone is nontoxic. Therefore it appears that an R_2 group is necessary for toxicity.

Since all three benzimidazole fungicides are toxic to the same fungi, the mechanism of action for the three would appear to be similar. Bartels & MacNeill (2) reported that a mutant of *Fusarium oxysporum* resistant to either benomyl or thiabendazole was also resistant to all three compounds; however, they found that a mutant resistant to furidazol was not resistant to benomyl or thiabendazole, and concluded that all three compounds have one mechanism of toxicity in common; but benomyl and thiabendazole must also have a common second mechanism of action which furidazol does not have.

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