J. Amer. Soc. Hort. Sci. 104(3):333–335. 1979.

Resistance to Thiabendazole and Benomyl of *Penicillium digitatum* and *P. italicum* Isolated from Citrus Fruit from Several Countries[1]

R. E. McDonald, L. A. Risse[2], and B. M. Hillebrand

USDA-SEA-AR European Marketing Research Center, Marconistraat 38 b, Rotterdam, The Netherlands

Additional index words. green mold, blue mold, *Citrus* sp.

Abstract. Strains of *Penicillium digitatum* (Sacc.) and *P. italicum* (Wehmer) resistant to thiabendazole and benomyl were isolated from decaying citrus fruits obtained from the Rotterdam, Netherlands, terminal market and originating from 18 countries. Significantly more *Penicillium* sp isolates with resistance to thiabendazole and benomyl were collected from grapefruit and lemons than from oranges. Significantly more isolates of *P. digitatum* than *P. italicum* grew on agar plates with 4, 10, or 40 ppm thiabendazole. A greater percentage of *P. digitatum* than *P. italicum* isolates grew on 4 and 10 ppm benomyl-agar plates, but a greater percentage of *P. italicum* than *P. digitatum* isolates grew on 40 and 80 ppm benomyl-agar plates. Both species were more resistant to thiabendazole than to benomyl, and often showed cross-resistance to the fungicides. Resistant *Penicillium* sp isolates produced larger colonies on 4 and 10 ppm thiabendazole and 40 and 80 ppm benomyl.

The successful marketing of citrus fruits in overseas markets greatly depends on the use of fungicides to control postharvest diseases that develop during transport and storage. Although several fungi cause citrus fruits to decay after harvest, green mold, *Penicillium digitatum,* is the major decay of citrus fruits from all production areas. Blue mold, *P. italicum,* is similar in distribution and development, but is observed less frequently than green mold (1).

Effective control of postharvest green and blue molds of citrus fruits has been advanced in recent years through the development of the benzimidazole fungicides, 2-(4-thiazolyl)-benzimidazole (thiabendazole) and methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl). Postharvest applications of thiabendazole or benomyl have been standard treatments for control of decay on citrus fruits in many producing areas since the early 1970's (except on fruit for export to countries that prohibit their use). Strains of *P. digitatum* and *P. italicum* resistant to one or more benzimidazole fungicides have been collected in citrus packinghouses in California (4), Israel (2), Florida (8), Japan (5) and Australia (7). Benzimidazole-resistant isolates of *Penicillium* sp have also been found in orchards and in packinghouses where benzimidazoles have never been used (4, 5), indicating that resistant strains occur naturally. The occurrence of both green and blue penicillia with resistance to benzimidazoles is a potentially serious situation. Past studies (2, 4, 5, 7, 8, 9) have demonstrated the existence of resistant molds, but have not shown the extent to which these resistant molds occur on infected fruit in overseas terminal markets.

The present investigation was carried out to determine actual frequencies of resistant *P. digitatum* and *P. italicum* on decaying fruit from different countries of origin and to determine the differential resistance of these molds to various levels of thiabendazole and benomyl.

Materials and Methods

Consignments of grapefruit, orange, and lemon fruits were sampled and spore masses collected weekly over a period of 1 year (1976) from warehouses and a wholesale market in Rotterdam, The Netherlands. No more than 3 decaying fruit of one fruit class from any one packinghouse (as identified on the container) were sampled in a consignment or shipment in order to obtain diversity in the samples collected.

Spore masses collected from decaying fruits were classified as either *P. italicum* or *P. digitatum* by color, and the identifications were verified in culture by color and by the distinctive coremia that are produced by *P. italicum,* but not by *P. digitatum.*

Spore masses from decaying fruits were collected on sterile cotton swabs, which were placed in individual plastic tubes and taken to the laboratory. Two ml of sterile distilled water was added to each tube, which was then vibrated on a Vortex mixer to separate the spore masses. Petri dishes (90 mm diameter) containing 10 ml of fungicide-agar were inoculated, in the center, with a 3-mm loopful of the suspension. The plates were held at 28°C, and the diameter of colonies was measured 1 week after inoculation.

The fungicide-agar used was made as follows: Difco potato dextrose agar, 19.5 g; Difco yeast extract, 0.5 g; and distilled water, 480 ml. To avoid degrading the benomyl by autoclaving, it was added after the agar was autoclaved and cooled to about 55°C. Enough thiabendazole or benomyl to result in concentrations of 4, 10, 40, and 80 ppm of the fungicide was dissolved in 20 ml of 70% ethanol and added to the agar before pouring the plates. Twenty ml of 70% ethanol was mixed with the agar to make the control plates. These concentrations of the fungicides were selected because they are in the range of or exceed the currently allowed residue levels for thiabendazole (10 ppm) and for benomyl [3.5 ppm expressed as methyl 2-benzimidazolecarbamate (MBC)] for citrus fruit in The Netherlands (6).

Results and Discussion

Recovery of fungicide-resistant isolates. In this paper, resistance refers to the ability of a mold to grow in the presence of a fungicide(s).

A total of 1793 isolates of either *P. digitatum* or *P. italicum* were obtained from decaying grapefruit, lemons, and oranges from 18 countries (Table 1); 83% were *P. digitatum* and the remainder were *P. italicum.* Fruits from the United States were identified according to the producing areas of California-Arizona, Florida, and Texas. Benzimidazole-resistant penicillia were responsible for nearly half the decay of fruit sampled (Table 2).

[1]Received for publication October 22, 1978. Mention of a trademark or brand name is for identification only and does not constitute endorsement by the U.S. Department of Agriculture over others which may also be suitable.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.

[2]Present address: USDA-SEA-AR, 2120 Camden Road, Orlando, FL 32803.

Table 1. Percentage of isolates of *Penicillium digitatum* (green mold) and *P. italicum* (blue mold) growing on potato dextrose agar with different concentrations of thiabendazole and benomyl.

	No. of isolates		Thiabendazole								Benomyl							
			4 ppm		10 ppm		40 ppm		80 ppm		4 ppm		10 ppm		40 ppm		80 ppm	
Origin	Green	Blue	Green	Blue	Green	Blue	Green	Blue	Green	Blue	Green	Blue	Green	Blue	Green	Blue	Green	Blue
Algeria	27	3	37	0	37	0	30	0	15	0	30	0	22	0	0	0	0	0
Argentina	142	32	49	22	47	25	46	22	31	13	39	25	13	19	4	3	3	0
Australia	2	8	100	38	100	38	100	38	50	0	100	38	50	38	0	25	0	25
Brazil	86	15	33	27	24	27	24	27	14	20	19	27	15	20	5	7	4	7
California (U.S.)	388	62	55	48	50	39	49	35	24	31	50	37	46	24	3	2	2	0
Chile	13	2	15	0	8	0	8	0	8	0	8	0	8	0	0	0	0	0
Cuba	19	2	37	0	37	0	37	0	16	0	26	0	26	0	16	0	11	0
Cyprus	50	30	14	7	12	7	12	3	6	0	12	7	10	0	6	0	0	0
Egypt	22	21	27	33	18	33	18	33	14	29	14	19	0	10	0	5	0	5
Florida (U.S.)	65	1	28	0	17	0	17	0	3	0	15	0	6	0	2	0	2	0
Greece	57	3	16	67	11	67	9	67	7	33	9	67	7	33	0	33	0	33
Honduras	24	0	54	–	39	–	39	–	25	–	29	–	21	–	7	–	7	–
Israel	99	28	90	86	89	82	89	82	71	79	80	86	66	61	18	39	12	32
Italy	73	5	49	60	48	60	44	60	21	60	44	60	42	60	16	20	15	20
Morocco	56	12	57	33	54	33	52	33	30	25	43	33	32	33	16	25	16	25
South Africa[z]	115	26	77	81	77	73	73	73	23	50	32	62	22	62	18	38	13	31
Spain	146	31	31	29	30	13	26	10	8	0	16	13	12	13	3	3	3	3
Texas (U.S.)	57	6	35	17	30	17	28	17	14	17	26	17	26	17	4	0	4	0
Turkey	7	1	29	100	14	100	14	100	0	0	14	100	14	0	0	0	0	0
Uruguay	43	14	58	64	55	57	37	64	30	64	51	64	35	21	28	14	23	14
Totals	1491	302																
Means			48.4	42.1	44.7	37.7	42.5	36.1	22.5	27.8	36.5	35.8	28.9	25.8	7.3	11.6	5.6	9.6

[z]Includes Swaziland.

Table 2. Percentage of isolates of *Penicillium digitatum* and *P. italicum* from decaying grapefruit, lemons, and oranges growing on potato dextrose agar containing 4 ppm thiabendazole or benomyl.

	Grapefruit		Lemon		Orange	
Penicillium sp.	No. isolates	% resistant	No. isolates	% resistant	No. isolates	% resistant
P. digitatum	344	50.9	428	53.0	719	46.3
P. italicum	59	49.2	55	56.4	188	37.8
Totals	403		483		907	
Means		50.6a[z]		53.4a		44.5b

[z]Mean separation by Duncan's multiple range test, 1% level.

More of the isolates of *P. digitatum* from grapefruit and oranges grew on benzimidazole-agar than did isolates of *P. italicum.* Resistant *P. italicum* isolates were more commonly isolated from lemons (Table 2). Resistant isolates of *Penicillium* sp. were recovered more frequently from grapefruit and lemons than from oranges (Table 2).

Differential resistance of green and blue molds to benzimidazoles. The percentages of *Penicillium* sp. growing on 4, 10, 40, and 80 ppm thiabendazole- and benomyl-agar plates are presented in Table 1. Significantly ($P<5\%$) more *P. digitatum* than *P. italicum* isolates grew on thiabendazole plates, except at the 80 ppm level. A greater percentage of *P. digitatum* isolates grew on benomyl plates at the 4 and 10 ppm levels but not at the 40 and 80 ppm levels, but the difference was not significant. The more frequent occurrence of benzimidazole resistance among isolates of *P. digitatum* than among isolates of *P. italicum* is in contrast with other findings (1, 3, 4) and can be attributed to the large number of isolates from many origins. Increasing concentrations of thiabendazole and benomyl resulted in the growth of a correspondingly decreasing number of resistant isolates, indicating that there are degrees of sensitivity and resistance (Table 1), as were found by other investigators (4, 5).

Colony diameters of both *Penicillium* sp. were significantly ($P<5\%$) larger on agar containing 4 or 10 ppm thiabendazole than they were on agar containing 4 or 10 ppm benomyl (Table 3). The reverse was true at concentrations of 40 or 80 ppm where growth was best on benomyl.

Significantly ($P<1\%$) more of the collected isolates grew on agar plates with 4 ppm thiabendazole (45.9%) than on plates with 4 ppm benomyl (35.8%). This indicates that the isolates tested are more resistant to thiabendazole at this level of concentration as compared to benomyl. Harding (4) also found that resistant-strains of *Penicillium* sp. were more resistant to thiabendazole. Wild and Rippon (9) found a strain of *P. digitatum* and Muirhead (7) found 2 strains of *P. italicum* that were more resistant to benomyl than to thiabendazole.

The penicillia often expressed cross-resistance to thiabendazole and benomyl: 78% of the isolates that grew on 4 ppm thiabendazole also grew on 4 ppm benomyl. Cross-resistance would be expected because of structural resemblance of the fungicides. These findings parallel earlier work (4, 8).

Occurrence of resistant green and blue molds. The resistance of *P. digitatum* and *P. italicum* to benzimidazoles is a common

Table 3. The effect of different concentrations of thiabendazole and benomyl in potato dextrose agar on growth of *Penicillium digitatum* (green mold) and *P. italicum* (blue mold).

Fungicide	Concn (ppm)	Mold growth diameter (% of control)	
		Green	Blue
Thiabendazole	4	86[z]	84
	10	70	65
	40	31	27
	80	20	19
Benomyl	4	49	42
	10	31	31
	40	41	31
	80	38	37

[z]Differences in growth within columns at similar concentrations of fungicides are valid at the 5% level.

problem in most citrus producing countries and the situation may be more serious in some countries than in others. For example, high percentages of resistant isolates were collected from Israeli and South African fruits and comparatively low percentages from Cypriot and Greek fruits. The differences in percentages of resistant fungi between countries may be attributed to different sanitation practices; recycling of resistant strains in packinghouses and in storage; and the selection of resistant strains through preharvest benomyl sprays, natural selection, and other factors.

It appears that successful control of postharvest decays of citrus probably needs to involve fungicides other than the benzimidazoles. No worthwhile practical levels of decay control can be expected by increasing fungicide concentrations.

Literature Cited

1. Eckert, J. W., and M. J. Kolbezen. 1971. Chemical treatments for the control of postharvest diseases of citrus fruits. *Proc. 6th Br. Insectic. Fungic. Conf.* 3:683-693.
2. Gutter, Y. 1973. Benzimidazole-resistant strains of citrus fruit pathogens. Div. of Fruit and Veg. Storage, Research Summaries 1971-73. The Volcani Center, Israel. p. 56-57.
3. ———. 1975. Interrelationship of *Penicillium digitatum* and *P. italicum* in thiabendazole-treated oranges. *Phytopathology* 65: 498-499.
4. Harding, P. R. 1972. Differential sensitivity to thiabendazole by strains of *Penicillium italicum* and *P. digitatum. Plant Dis. Rptr.* 56:256-260.
5. Kuramoto, T. 1976. Resistance to benomyl and thiophanate-methyl in strains of *Penicillium digitatum* and *P. italicum* in Japan. *Plant Dis. Rptr.* 60:168-172.
6. McDonald, R. E., and T. B. O'Connell. 1977. Citrus fungicide residues and legislation in western European countries. *Proc. Int. Soc. Citriculture* 1:296-298.
7. Muirhead, I. F. 1974. Resistance to benzimidazole fungicides in blue mould of citrus in Queensland. *Austral. J. Expt. Agr. Anim. Husb.* 14:698-701.
8. Smoot, J. J., and G. E. Brown. 1974. Occurrence of benzimidazole-resistant strains of *Penicillium digitatum* in Florida citrus packinghouses. *Plant Dis. Rptr.* 58:933-934.
9. Wild, B. L., and L. E. Rippon. 1975. Response of *Penicillium digitatum* strains to benomyl, thiabendazole, and sodium O-phenylphenate. *Phytopathology* 65:1176-1177.

J. Amer. Soc. Hort. Sci. 104(3):335–338. 1979.

Light Compensation Point and Leaf Distribution of *Ficus benjamina* as Affected by Light Intensity and Nitrogen—Potassium Nutrition[1]

C. R. Johnson[2], J. K. Krantz[2], J. N. Joiner[2], and C. A. Conover[3]
Department of Ornamental Horticulture, University of Florida, Gainesville, FL 32611

Additional index words. acclimatization, foliage plants.

Abstract. **Plants of *Ficus benjamina* L. had less leaves in the upper half of sun and shade-grown plants with higher N rates. Potassium affected leaf distribution only in bottom portion of canopy and had no effects on LCP. High N levels increased light compensation point (LCP) of sun-grown plants, but reduced LCP of shade-grown plants. Increased number of leaves in upper half of sun-grown plants reduced LCP but shade-grown plants showed an opposite effect.**

Ficus benjamina, a tropical tree used widely for interior decoration, often drops many leaves when moved from high to low light intensities (8, 9). Such leaf drop may be caused by interior light intensities below plants' LCP. Relationships between light intensities and photosynthesis have been studied by many researchers who developed light intensity vs. apparent photosynthesis curves for a wide range of plants (2, 4, 5, 7, 14, 17). Plants grown in sun generally showed high light saturation and LCP than shade-grown plants. Light intensity and fertilization during production of *F. benjamina* have been shown to affect LCP (6) and plant adaptability to low light conditions (15).

Horn (12) suggested that shape and distribution of leaves in multilayered tree canopies affected LCP, especially of lowest leaves. Plant genotype (18), planting densities (16) and light intensities (13) have been shown to affect leaf distribution in tree canopies but cultural inputs such as fertilization have not been considered. Objectives were to determine effects of N-K ratios and light intensity on leaf distribution and plant LCP.

[1]Received for publication November 17, 1978. Florida Agricultural Experiment Stations, University of Florida. Journal Series No. 1401. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.

[2]University of Florida, Gainesville.

[3]Agricultural Research Center, Apopka, Florida.

[4]Soluble Trace Element Mix manufactured by Peters Co., Inc., Allentown, Pa.