Occurrence of *Penicillium digitatum* and *P. italicum* Resistant to Benomyl, Thiabendazole, and Imazalil on Citrus Fruit from Different Geographic Origins

VINCENT G. BUS, ANTON J. BONGERS, and LAWRENCE A. RISSE, Agricultural Research Service, U.S. Department of Agriculture, European Marketing Research Center, Marconistraat 38, 3029 AK Rotterdam, The Netherlands

ABSTRACT

Bus, V. G., Bongers, A. J., and Risse, L. A. 1991. Occurrence of *Penicillium digitatum* and *P. italicum* resistant to benomyl, thiabendazole, and imazalil on citrus fruit from different geographic origins. Plant Dis. 75:1098-1100.

In 1 yr, 1,287 isolates of *Penicillium digitatum* and *P. italicum* were collected from decayed mandarins (*Citrus reticulata*), oranges (*C. sinensis*), lemons (*C. limon*), and grapefruit (*C. paradisi*) at the wholesale markets in Rotterdam and Paris. The isolates were cultured on media with various fungicides and concentrations. Thirty-seven percent of the *P. digitatum* isolates grew at 4 and 10 mg/L of thiabendazole (TBZ), 35 and 34% grew at 4 and 10 mg/L of benomyl (BEN), respectively, and 17 and 12% grew at 0.2 and 0.5 mg/L of imazalil (IM), respectively. Ninety percent of the *P. digitatum* strains resistant to TBZ were also resistant to BEN, and 13% of the strains tolerant to either one or both benzimidazoles showed double resistance to IM. Forty percent of the *P. italicum* isolates showed resistance to 4 mg/L of TBZ and 38% to 10 mg/L of TBZ, but only 29 and 13% were resistant to BEN at 4 and 10 mg/L, respectively, and 1% to 0.2 mg/L of IM. The level of cross-resistance between the benzimidazoles was 73%. Resistance of *Penicillium* to the benzimidazoles was highest in isolates taken from grapefruit, less in those from oranges and mandarins, and least in isolates recovered from lemons. However, the level of resistance to IM was higher in isolates from lemons.

Additional keywords: blue mold, green mold, postharvest fungicides

After 20 yr of intensive use, the fungicides thiabendazole (TBZ) and benomyl (BEN) are still widely applied in postharvest treatments of citrus fruit, except for in the United States where BEN has been withdrawn by the manufacturer for postharvest use on food crops. With the introduction of the benzimidazole compounds in the early 1970s, citrus packers had powerful means to control green and blue mold decay, which at that time appeared to have developed some resistance to biphenyl and ortho-phenylphenol (OPP) (15).

Research has shown that TBZ and BEN are very effective in controlling decay and sporulation caused by *Penicillium digitatum* (Pers.:Fr.) Sacc. and *P. italicum* Wehmer (12,13). However, within a few years after their introduction, many cases of resistance were reported from different countries (14). Development of resistance to the benzimidazole fungicides and multiple resistance was described in detail by Houck (10). Also in this review, alternatives were recommended to prevent or control the development of fungicide-resistant strains, such as avoiding injuries during harvesting, packing, and transporting; changing packinghouse and storage room layouts and sanitation procedures; and using different types of fungicides alternately or simultaneously.

A new type of fungicide became available to citrus packers with the introduction of imazalil (IM) in the early 1980s. As an experimental fungicide, the imidazole compound had shown good control of *Penicillium* decay and sporulation (16) and control of strains resistant to the benzimidazoles (9). IM inhibits ergosterol biosynthesis by inhibiting the demethylation of lanosterol (11). The chances of resistance to this type of fungicide developing were expected to be fairly low (11,16). However, a few years after resistance of *P. italicum* to ergosterol biosynthesis inhibitors (EBI) was induced under laboratory conditions (6), the incidental occurrence of resistant strains in the packinghouses was reported (3,7).

In this paper, the results of a survey on the current distribution of resistance of *P. digitatum* and *P. italicum* to TBZ, BEN, and IM are presented. These three compounds, along with biphenyl and OPP, are the most commonly used fungicides in postharvest treatments of citrus in most citrus-producing areas.

MATERIALS AND METHODS

For 1 yr, once every month, spores of green and blue mold were collected from citrus fruit at the wholesale markets in Rotterdam (Spaanse Polder, October 1988–September 1989) and Paris (M.I.N. de Rungis, November 1988–October 1989). The spores were collected from a maximum of three decayed mandarins (*Citrus reticulata* Blanco), oranges (*C. sinensis* (L.) Osbeck), lemons (*C. limon* (L.) N. L. Burm.), or grapefruit (*C.* × *paradisi* Macfady.) per fruit lot with sterile cotton swabs that were stored in sterile test tubes. The *Penicillium* species of the isolates was determined by sight. In cases where further identification was needed, the isolates were sent to Microbial/Plant Technology Research, USDA-ARS, New Orleans, LA, or Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Isolates other than *P. digitatum* or *P. italicum* were excluded from the study.

In the laboratory, 2 ml of sterile distilled water was added to the tube and vibrated on a vortex mixer to bring the spores into suspension. With a 3-mm loop, a droplet (6 μl) of the spore suspension ($\pm 10^7$ spores per milliliter) was placed on potato-dextrose agar in three-cell petri dishes (9 cm), each cell containing 3.3 ml of agar. The agar was amended after autoclaving with 0, 4, or 10 mg/L of TBZ (Sigma Chemical Co., St. Louis, MO) or BEN (Agrichem B.V., Oosterhout, The Netherlands) or 0, 0.2, or 0.5 mg/L of IM (Janssen Pharmaceutica, Beerse, Belgium), all from 500× concentrated stock solutions in 25% (v/v) aqueous ethanol. They were the same concentrations McDonald et al (14) used in their resistance study. The lower (0.2 mg/L) concentration of IM was two to three times the ED_{50} level of IM-sensitive strains (H. M. Hoogendoorn, *unpublished*) (2). Growth (the visible formation of a colony with, in most cases, spores) was assessed after 5 days of incubation at 24 C. In this study, isolates exhibiting any growth were defined as resistant. At the same time, pathogenicity of the isolates was tested by making three wounds in an orange with a 6-mm corer and wetting the wounds with a cotton swab saturated with spore suspension. Beforehand, the oranges were disinfested by dipping in alcohol, followed by dipping in a 1% hypochlorite + 1% Tween 20 solution for 1 min, and then rinsed thoroughly in running tap water. Development of decay was assessed after 7 days

Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Accepted for publication 8 April 1991.

Table 1. Percentage of *Penicillium digitatum* and *P. italicum* isolates by geographic origin showing growth on potato-dextrose agar amended with thiabendazole (TBZ), benomyl (BEN), or imazalil (IM)

	P. digitatum							*P. italicum*						
	No. of	TBZ (mg/L)		BEN (mg/L)		IM (mg/L)		No. of	TBZ (mg/L)		BEN (mg/L)		IM (mg/L)	
Origin	isolates	4	10	4	10	0.2	0.5	isolates	4	10	4	10	0.2	0.5
Argentina	115	69	70	66	63	1	0	33	58	58	54	36	0	0
Brazil	31	19	19	10	10	0	0	3	100	100	100	67	0	0
California	61	75	75	77	75	54	15	25	20	20	20	20	0	0
Cuba	10	0	0	0	0	0	0	4	75	75	25	0	0	0
Cyprus	5	60	60	60	60	20	0	12	75	75	67	0	0	0
Florida	75	81	81	83	83	1	1	1	100	100	100	100	0	0
Greece	17	18	18	24	24	18	0	11	18	9	0	0	0	0
Israel	4	100	100	100	100	25	25	7	86	86	57	0	0	0
Italy	40	8	8	8	5	3	0	14	0	0	0	0	0	0
Morocco	115	30	30	30	30	2	1	99	27	25	20	5	2	0
South Africa	33	61	61	18	18	0	0	24	83	83	50	21	0	0
Spain	380	19	18	18	18	31	26	57	37	33	26	5	0	0
Tunisia	27	11	11	11	7	0	0	6	33	33	17	17	0	0
Turkey	18	17	17	17	17	0	0	11	27	27	18	9	9	0
Uruguay	27	59	59	59	59	4	0	22	45	45	32	27	5	0
Total	958	37	37	35	34	17	12	329	40	38	29	13	1	0

of incubation at room temperature in plastic bags.

RESULTS

In total, 1,287 isolates of green and blue mold were collected from citrus fruit originating from 14 countries (Table 1). Within the United States, California and Florida were distinguished separately as production areas. Forty-eight percent of the isolates showed in vitro resistance to one or more fungicides. Thirty-seven percent of the *P. digitatum* isolates showed growth on 4 and 10 mg/L of TBZ, but resistance to BEN appeared to be lower—35% at 4 mg/L and 34% at 10 mg/L. The level of cross-resistance, resistance to one or more related compounds, was high: 90% of *P. digitatum* strains resistant to TBZ were also resistant to BEN. Only 17 and 12% of the *P. digitatum* isolates showed growth on agar amended with 0.2 and 0.5 mg/L of IM, respectively. Some isolates showed double resistance; 13% of the strains resistant to one or both benzimidazole compounds showed growth in the presence of IM. About 39% of the *P. italicum* isolates collected showed resistance to TBZ at 4 and 10 mg/L, but only 29 and 13% were resistant to BEN at 4 and 10 mg/L, respectively. The level of cross-resistance was 73%. The incidence of blue mold isolates resistant to 0.2 mg/L of IM was very low (1%), and none of the strains showed growth at 0.5 mg/L. Two percent of the blue mold strains resistant to the benzimidazoles showed double resistance to IM. The percentage of resistant isolates to the benzimidazoles by origin was in the range of 0–100% for both *P. digitatum* and *P. italicum*. For IM, most countries showed lower numbers of resistance. However, there were two exceptions with *P. digitatum*—California and Spain (Table 1). In the case of California, the level of double resistance was very high (68%) compared with 12% for Spain.

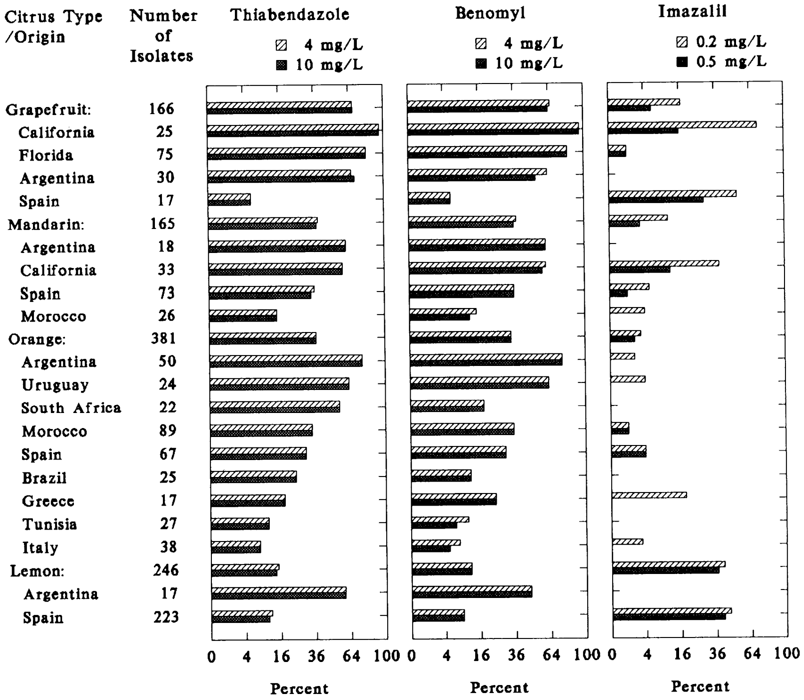

Fig. 1. Percentage of *Penicillium digitatum* isolates showing growth on potato-dextrose agar amended with thiabendazole, benomyl, or imazalil by citrus type and geographic origin (for the origins with more than 10 isolates per citrus type).

Isolates of *P. digitatum* and *P. italicum* with resistance to TBZ or BEN (Figs. 1 and 2) were more prevalent on grapefruit (68%), less on oranges and mandarins, and least on lemons. However, the percentage of *P. digitatum* strains originating from lemons resistant to IM was high (41 and 37% at 0.2 and 0.5 mg/L, respectively) compared with the other citrus types. All of the isolates collected were pathogenic to oranges. However, no relation in the presence of resistance and pathogenicity was found.

DISCUSSION

The overall percentages of resistant isolates were found to be lower for TBZ and at similar levels for BEN with *P. digitatum*, and similar for TBZ and lower for BEN with *P. italicum*, compared with the findings of McDonald et al (14). The number of isolates resistant to BEN was lower compared with TBZ, because this compound generally is more active than TBZ at the same concentrations. This effect was more clear with blue mold than with green mold (Figs. 1 and 2). A decrease in resistance to the benzimidazole compounds can be attributed to effective resistance management (1), of which sanitation of the packinghouse with multisite fungicides forms the basis (8). Increasing the application levels of TBZ or BEN is not effective once resistance

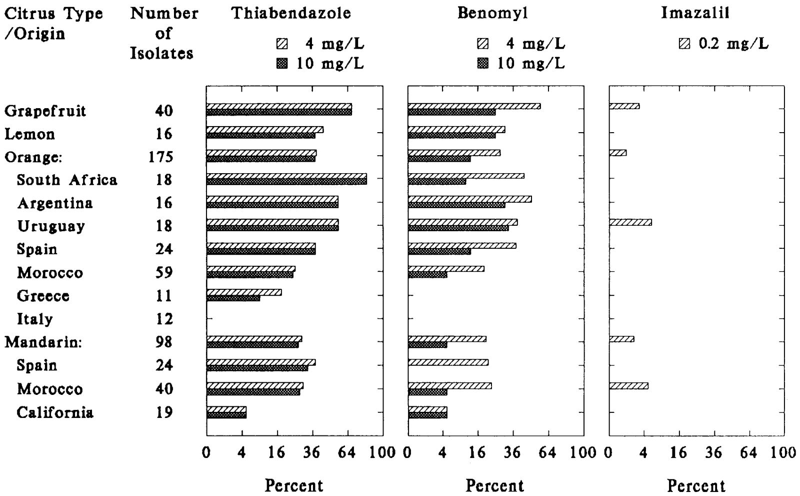

Fig. 2. Percentage of *Penicillium italicum* isolates showing growth on potato-dextrose agar amended with thiabendazole, benomyl, or imazalil by citrus type and geographic origin (for the origins with more than 10 isolates per citrus type).

occurs, because mutation to high levels of resistance takes place in one single step.

To reduce selection pressure, it is advised to apply benzimidazole fungicides at reduced but effective rates (4,5) and use it either simultaneously or alternately with nonrelated fungicides (18). However, in the situation of double resistance, the alternative fungicide can maintain or even increase the frequency of isolates resistant to the benzimidazoles, as is shown with OPP, 2-AB, and guazatine (8,19). The same can be expected from IM, as shown by California (Fig. 1), where the level of double resistance was 68%. The process of directional selection leading to resistance with IM is stimulated by inadequate applications of the fungicide attributable to excessive fruit flow on the packing line, resulting in minor coverage of and lower concentrations on the fruit, and by the effect of time (i.e., during storage) after treatment with IM. This process can be reversed again by effective resistance management, partly because strains resistant to IM are less competitive in infectivity than sensitive strains (3,17). Resistance levels of *P. italicum* to IM were very low because this fungus occurs less frequently than *P. digitatum*, especially on lemons and grapefruit (Fig. 2).

The use of the benzimidazole fungicides in *Penicillium* decay control is limited, depending on the magnitude of the resistance problem. IM is used as the alternative fungicide in benzimidazole resistance management in most citrus-producing areas. Favorable results in *Penicillium* decay control have also been reported with other demethylation-inhibiting fungicides (9,16), but like with the benzimidazoles, resistance management with these fungicides is also necessary. (Cross-)resistance can be expected (6), and as shown by Wild (19) and confirmed by this study, double resistance can develop.

More research on new fungicides and the development of fungicides with a different mode of action is necessary. The resistance potential of these compounds and the resistance levels of compounds already in use must be assessed and monitored continually for decision-making on their application. At the same time, the use of resistant citrus varieties and alternative nonfungicidal methods of decay control must be stimulated to accommodate the growing concern of the general public and the growers on the use of chemicals on foods.

LITERATURE CITED

1. Bancroft, M. N., Gardner, P. D., Eckert, J. W., and Baritelle, J. L. 1984. Comparison of decay control strategies in California lemon packinghouses. Plant Dis. 68:24-28.
2. Brown, G. E. 1989. Baseline sensitivity of Florida isolates of *Penicillium digitatum* to imazalil. Plant Dis. 73:773-774.
3. Dave, B., Sales, M., and Walia, M. 1989. Resistance of different strains of *Penicillium digitatum* to imazalil treatment in California citrus packinghouses. Proc. Fla. State Hortic. Soc. 102:178-179.
4. Delp, C. J. 1988. Resistance management strategies for benzimidazoles. Pages 41-43 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
5. De Waard, M. A. 1988. Interactions in fungicide combinations. Pages 98-100 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
6. De Waard, M. A., Groeneweg, H., and van Nistelrooy, J. G. M. 1982. Laboratory resistance to fungicides which inhibit ergosterol biosynthesis in *Penicillium italicum*. Neth. J. Plant Pathol. 88:99-112.
7. Eckert, J. W. 1987. *Penicillium digitatum* biotypes with reduced sensitivity to imazalil. (Abstr.) Phytopathology 77:1728.
8. Eckert, J. W. 1988. Dynamics of benzimidazole-resistant *Penicillia* in the development of postharvest decays of citrus and pome fruits. Pages 31-35 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
9. Eckert, J. W., Bretschneider, B. F., and Ratnayake, M. 1981. Investigations on new postharvest fungicides for citrus fruits in California. Proc. Int. Soc. Citric. 2:804-810.
10. Houck, L. 1977. Problems of resistance to citrus fungicides. Proc. Int. Soc. Citric. 1:263-269.
11. Köller, W., and Scheinpflug, H. 1987. Fungal resistance to sterol biosynthesis inhibitors: A new challenge. Plant Dis. 71:1066-1074.
12. McCornack, A. A., and Brown, G. E. 1967. Thiabendazole, an experimental fungicide for fresh citrus fruit. Proc. Fla. State Hortic. Soc. 80:232-237.
13. McCornack, A. A., and Brown, G. E. 1969. Benlate, an experimental postharvest citrus fungicide. Proc. Fla. State Hortic. Soc. 82:235-238.
14. McDonald, R. E., Risse, L. A., and Hillebrand, B. M. 1979. Resistance to thiabendazole and benomyl of *Penicillium digitatum* and *P. italicum* isolated from citrus fruit from several countries. J. Am. Soc. Hortic. Sci. 104(3):333-335.
15. Smoot, J. J., and Winston, J. R. 1967. Biphenyl-resistant citrus green mold reported in Florida. Plant Dis. Rep. 51:700.
16. Tuset, J. J., Piquer, J., Garcia, J., Martinez, J. M., and Roca, M. 1981. Activity of imidazole fungicides to control postharvest citrus decay. Proc. Int. Soc. Citric. 2:784-787.
17. Van Gestel, J. 1988. Imazalil-sensitive and less sensitive strains of *Penicillium digitatum*: Competition in oranges. Proc. Int. Soc. Citric. 6:1511-1514.
18. Wade, M. 1988. Strategies for preventing or delaying the onset of resistance to fungicides and for managing resistance occurrences. Pages 14-15 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
19. Wild, B. L. 1983. Double resistance by citrus green mould *Penicillium digitatum* to the fungicides guazatine and benomyl. Ann. Appl. Biol. 103:237-241.