FUNGICIDAL TOLERANCE OF BOTRYTIS WITHIN COLORADO GREENHOUSES

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Dichloran was the only fungicide that was completely inhibitory to all *Botrytis* isolates tested from containerized conifer seedlings in three Colorado greenhouses.

Botrytis cinerea Pers. ex Fr. is an important pathogen on numerous crops grown within greenhouses (*4*). Recent investigations at the Colorado Hydroponics Nursery in Lyons, Colo. indicated widespread mortality of containerized lodgepole pine (*Pinus contorta* Dougl.) in greenhouses caused by the pathogen. Biweekly benomyl application through overhead sprinkling systems did not control the disease.

Previous reports (*2, 4, 6*) have identified benomyl-tolerant *Botrytis* strains. Tests were conducted to determine if benomyl-tolerant strains of the fungus occurred within Colorado nurseries where the chemical was routinely used. In order to provide growers with alternative fungicide options, other commonly used fungicides were also tested for tolerance by *Botrytis.*

Materials and Methods

Botrytis was isolated from containerized conifer (lodgepole pine, Scots pine (*Pinus sylvestris* L.), Engelmann spruce (*Picea engelmannii* Parry), and blue spruce (*Picea pungens* Engelm.)) seedlings in three Colorado nurseries: Colorado Hydroponics (Lyons), Colorado State Forest Service Nursery (Fort Collins), and the Forest Service Mt. Sopris Tree Nursery (Carbondale). Two isolates from California redwood (*Sequoia sempervirens* D. Don) (received from A. H. McCain), one known to be tolerant to benomyl and the other known to be benomyl-sensitive, were used for reference.

Tolerance of fungicides was determined by assessing radial growth of *Botrytis* isolates on the surface of potato dextrose agar (PDA) amended with fungicides. Seven fungicides (table 1), mixed in PDA at 50 parts per million (ppm) active ingredient (a.i.) were used to test *Botrytis* tolerance. Isolates tested included seven from Colorado Hydroponics and benomyl-sensitive (78-18) and tolerant (78-19) isolates from California. A second growth test assessed responses of *Botrytis* to three fungicides (benomyl, dichloran, and chlorothalonil) at concentrations ranging from 5 to 500 ppm a.i. Three isolates, chosen for their range of benomyl tolerance from each of the three nurseries, plus the two California reference isolates were evaluated.

Effects of benomyl on *Botrytis* spore germination were determined. Spores were added to PDA amended with 50 ppm of the fungicide and checked for germination after 24 hours' incubation.

Results

Results of the first growth test (table 2) indicated a range of benomyl tolerance for the *Botrytis* isolates tested. Two isolates (78-11, 78-12) showed high levels of tolerance, two (78-1, 78-13) were intermediate in response, and three (78-4, 78-6, 78-7) were sensitive as evidenced by little growth over the benomyl-amended agar. Response to the two types of benomyl used (Dupont and Fertilome) was significantly different in five of the seven Colorado isolates tested.

Of all the fungicides tested, dichloran was the only one for

Table 1.—*Fungicides used to test tolerance of* Botrytis *isolates.*

Chemical	Trade name	Manufacturer
1. benomyl	Benlate® 50 WP	Dupont
2. benomyl	Benomyl®	Fertilome
3. dichloran	Botran® 75 WP	Tuco (Upjohn)
4. captan	Captan® 50 WP	Stauffer
5. chlorothalonil	Daconil 2787®	Diamond Shamrock
6. mancozeb	Dithane® M-45	Rohm and Hass
7. zineb	Dithane® Z-78	Rohm and Hass

Table 2.—*Fungicidal effects on radial growth of* Botrytis[1]

Fungicide Treatment	*Botrytis* isolates California[2] 78–18	78–19	Colorado hydroponics 78–1	78–4	78–6	78–7	78–11	78–12	78–13
Check	100 A	100 A	100 A	100 A	100 A	100 A	100 A	100 C	100 A
Benomyl (Dupont)	1 C	91 A	17 BC	6 C	9 C	8 D	90 AB	98 C	46 BC
Benomyl (Fertilome)	3 C	72 B	31 B	40 B	41 B	40 B	82 AB	122 B	74 AB
Dichloran	0 C	1 D	0 D	0 C	0 C	0 D	0 D	0 F	0 D
Captan	17 B	67 B	8 CD	6 C	12 C	10 CD	9 D	16 E	8 CD
Chlorothalonil	62 B	53 C	23 BC	5 C	19 C	22 C	31 CD	71 D	7 CD
Mancozeb	51 B	99 A	47 A	47 B	49 B	40 B	62 BC	104 C	55 B
Zineb	101 A	101 A	96 A	96 A	99 A	97 A	62 BC	164 A	99 A

[1]Fungicide concentration = 50 ppm a.i.; growth expressed as percent of check. Within each isolate, means followed by the same capital letter are not significantly different (P = 0.05) using Tukey's test for multiple comparison of treatment means.
[2]California reference isolates: 78–18 = benomyl sensitive
78–19 = benomyl tolerant.

which no tolerance was shown (table 2). At least some degree of tolerance to all other fungicides was evident. Tolerance of individual isolates to more than one fungicide was common. The least effective fungicides were zineb and mancozeb; chlorothalonil was effective against only two isolates. Next to dichloran, captan was the most effective fungicide in limiting growth of *Botrytis.*

Results of the second growth test are represented graphically in figure 1 for three of the Colorado isolates tested. Increasing benomyl concentration resulted in greater sensitivity in all isolates tested. All isolates were sensitive to dichloran at all concentrations tested. Sensitivity to chlorothalonil generally increased with greater fungicide concentration.

Benomyl did not inhibit *Botrytis* spore germination. Germ tubes from spores of benomyl-sensitive isolates (determined from growth tests) often lysed or wrapped around the spore; whereas, germ-tubes from benomyl-tolerant isolates grew over the agar surface with no apparent inhibition.

Discussion

The development of tolerance to fungicides is mainly due to selection pressure exerted on resident populations of the pathogen by heavy applications of a single fungicide (*5*). When fungal populations are subjected to fungicides, sensitive individuals are selectively killed. Those individuals which harbor a mutation for tolerance may multiply without competition from the normal wild populations (*1*). This new, tolerant population then becomes dominant, and crop losses can occur despite continued fungicide application. Benomyl tolerance by *Botrytis* usually develops over time. However, tolerance existed within two Colorado nurseries even though benomyl had not been previously used. Tolerance either developed rapidly (within 1 month at Colorado Hydroponics and 1 year at Mt. Sopris) or resident *Botrytis* populations, which were sources of initial infection, were benomyl tolerant.

All isolates tested were sensitive

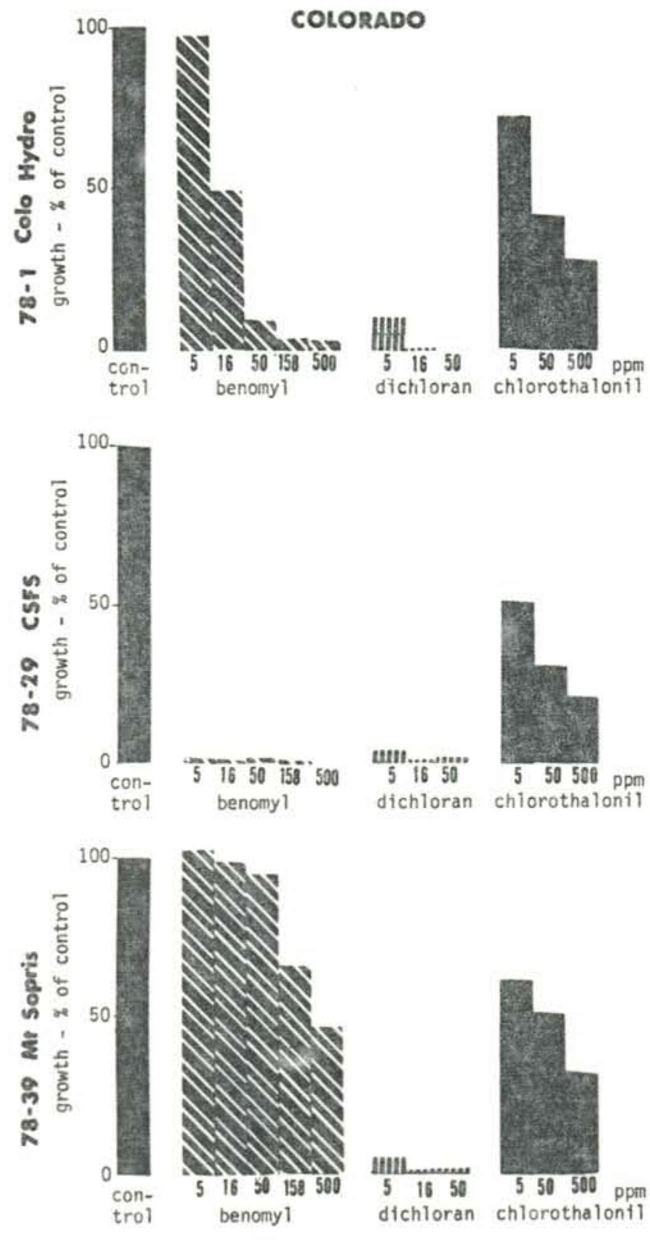

Figure 1.—*Growth response of three Colorado Botrytis isolates to varying concentrations of benomyl, dichloran, and chlorothalonil.*

to dichloran, even at low concentrations. This fungicide provides a possible alternative for *Botrytis* control in Colorado nurseries. However, use of a single fungicide is discouraged because the pathogen rapidly develops tolerance (*4*). Although chlorothalonil has been recommended as an alternative to benomyl for *Botrytis* control on containerized conifers (*3*), our results indicate that tolerance in the fungus populations tested may preclude its usefulness in certain Colorado nurseries. Captan was generally effective against the isolates tested. This nonsystemic organic compound has a history of good performance and is often considered an alternative to benomyl and other systemic fungicides for which tolerance may develop (*5*).

Fungicide-tolerant strains displayed decreasing tolerance with increasing fungicide concentration. However, from a practical standpoint, heavy fungicide applications will probably have little effect in overcoming tolerance. Such applications will most likely increase selection pressure and result in the development of more tolerant strains of the fungus.

Benomyl did not reduce spore germination of *Botrytis* isolates tested. Rather, the chemical affected germ tubes by causing lysis and abnormal growth in benomyl-sensitive isolates. Germ tube growth and development were not affected by benomyl in *Botrytis* strains tolerant to the chemical.

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