Strains of *Botrytis cinerea* Resistant to Benomyl and Captan in the Field

H. S. PEPIN, Research Scientist, and E. A. MacPHERSON, Technician, Agriculture Canada, Research Station, Vancouver, B.C. V6T 1X2

ABSTRACT

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Isolates of *Botrytis cinerea* from unsprayed and fungicide-treated field plots were resistant to captan and/or benomyl in laboratory tests. Benomyl-treated plots yielded significantly higher numbers of benomyl-resistant isolates than plots not treated with benomyl, but captan resistance was not related to captan treatment.

Additional key words: bean, *Fragaria ananassa*, fungicide resistance, *Phaseolus vulgaris*, raspberry, *Rubus idaeus*, strawberry

Recently, neither benomyl nor captan has given satisfactory control of *Botrytis cinerea* Pers. ex Fr. in the Fraser Valley of British Columbia, so a search for fungicide resistance was initiated.

Strains of *B. cinerea* resistant to benomyl have been isolated from many horticultural crops (1). Ogawa et al (3) list an extensive array of pathogenic fungi with field resistance or with developed laboratory resistance to a wide range of fungicides and bacteriocides, both systemic and nonsystemic. Resistance to systemic fungicides has become commonplace since the first report of tolerance of the cucumber powdery mildew fungus (*Sphaerotheca fuliginea* (Schlecht.) Poll.) to benomyl (5). Resistance to nonsystemics, however, is not so common, and many reports concern induced resistance in the laboratory, not the field (3). A biotype of *B. cinerea* capable of growing and sporulating in captan at 250,000 ppm was developed by laboratory manipulation (4). There are, however, no published reports on field development of this type of resistance. This paper contains just such a report.

MATERIALS AND METHODS

Isolates of *B. cinerea* were collected from a variety of sources from 1976 to 1979 (Table 1). In 1980, isolates were collected from a single bean field in Agassiz, B.C., containing rows that were unsprayed, treated with benomyl, or treated with captan and benomyl; they were also collected from a raspberry field in Abbotsford, B.C., containing unsprayed and captan-treated rows. The fungus was isolated on potato-dextrose agar (PDA) plates, maintained at room temperature, and subcultured by mass hyphal transfer as required. Within a few weeks after isolation, the cultures were screened for tolerance to benomyl (1976–1980) and captan (1978–1980). Four isolates that were resistant to either benomyl or captan in 1978 were retested in 1980 against both fungicides.

Commercial formulations of the fungicides were added to liquid PDA at 40 C before pouring into 100-mm petri plates. Concentrations of active ingredients were 0, 10, 100, 500, and 1,000 ppm. A 6-mm-diameter plug of mycelium from the edge of a young *B. cinerea* culture was placed, with the mycelium side down, in the center of each dish. Ten to 15 isolates were screened at a time, with three or four replicates of each. The cultures were incubated at room temperature for 2–4 days. When the fastest growing culture covered about two-thirds (1976, 1977) or all (1978–1980) of the plate, colony diameters were measured.

RESULTS

Sensitive *B. cinerea* strains did not grow on PDA plates containing benomyl at 10 ppm. However, many isolates, especially those from benomyl-treated sources, produced normal-looking but slower growing colonies (20–30% of control) on PDA containing benomyl at 1,000 ppm. Fifty-five percent of the isolates from sources treated with benomyl and captan-benomyl collected from 1976 to 1978 were benomyl resistant, compared with 14% of those from captan-treated and untreated plots (Table 1). This difference was highly significant ($P < 0.01$) by the chi-square test. Results from the 1980 tests with isolates from a single bean field showed the effect of chemical treatment on *B. cinerea* populations even more dramati-

Table 1. Sources of *Botrytis cinerea* isolates from field-grown crops and their reactions to benomyl and captan in fungicide resistance tests

Treatment Source	Isolates tested (no.)	Isolates resistant (no.) Benomyl (1,000 ppm)	Captan (100 ppm)	Captan (1,000 ppm)
Unsprayed				
1976 raspberry cane	5	0	...	...
1977 strawberry petiole	1	1	...	...
1978 strawberry fruit	14	1	4	2
1978 raspberry fruit	3	0	2	0
1978 bean pod	5	1	2	0
Benomyl[a]				
1976 strawberry petiole	55	36	...	...
1977 strawberry petiole	100	56	...	...
1978 raspberry cane	5	0	0	0
1978 bean pod	5	5	0	0
Captan[b]				
1978 bean pod	9	2	3	2
Captan-benomyl[c]				
1978 raspberry fruit	2	0	1	0
1978 raspberry cane	13	0	7	0
1978 bean pod	5	5	0	0
Unknown treatment				
1979 bean pod	19	4	4	2

[a] Strawberry and raspberry: 0.6 kg a.i./ha; four or seven applications. Bean: 1.25 kg a.i./ha; two applications.
[b] 1.75 kg a.i./ha; two applications.
[c] Raspberry: captan at 1.5 kg a.i./ha, benomyl at 0.3 kg a.i./ha; four applications. Bean: captan at 1.75 kg a.i./ha, benomyl at 0.35 kg a.i./ha; two applications.

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Table 2. Fungicide resistance in *Botrytis cinerea* isolates from a single field of beans, 1980

Treatment	Isolates tested (no.)	Isolates resistant (no.)[a]			
		Benomyl (1,000 ppm)	Captan (100 ppm)	Captan (1,000 ppm)	Benomyl (1,000 ppm) + captan (100 ppm)
Benomyl[b]	22	22 **	8 n.s.	1 n.s.	8 *
Captan-benomyl[c]	31	28 **	10 n.s.	2 n.s.	10 n.s.
Unsprayed	21	4	11	0	2

[a] ** = Different from unsprayed control by chi-square test at $P < 0.01$; * = different at $P < 0.05$; n.s. = not significant.
[b] 1.25 kg a.i./ha; two applications.
[c] Captan at 3.5 kg a.i./ha, benomyl at 0.7 kg a.i./ha; two applications.

cally: 100 and 90% of the isolates from rows treated with benomyl and captan-benomyl, respectively, were resistant to benomyl, as compared with 19% of those from untreated rows (Table 2).

Captan resistance was more difficult to find and define. Whereas many of the cultures appeared to thrive on PDA containing captan at 1,000 ppm, most of the growth did not penetrate the medium and could easily be lifted from the surface with a needle. Mycelia of some isolates managed to penetrate a few millimeters from the plug or in patchy patterns extending 1 or 2 cm over the medium; such isolates were considered resistant. After further incubation, these isolates often covered the plate. Two isolates from 1978 captan-treated beans, one from a 1979 bean of unknown treatment, and one from 1980 captan-treated raspberry displayed an unusually high degree of captan resistance on the medium containing captan at 1,000 ppm, reaching colony diameters that were 22% of the PDA control without the aforementioned superficial growth. Sporulation occurred with most isolates at all concentrations of fungicide used.

Thirty-eight percent of the isolates from sources treated with captan and captan-benomyl from the 1976–1978 collection (Table 1) showed some resistance to captan at 100 ppm, compared with 25% of those from benomyl-treated and untreated sources; however, the difference was not significant. Similarly, *B. cinerea* populations from unsprayed and captan-treated raspberry plots within the same field in 1980 did not differ significantly in captan resistance (*data not shown*), even when several criteria of resistance, including measures of superficial growth, were used.

Twenty-three benomyl-resistant *B. cinerea* isolates collected in 1980 showed some resistance to captan at 100 ppm: 3 were from unsprayed sources and 2, 8, and 10 were from sources treated with captan, benomyl, and captan-benomyl, respectively.

The one benomyl-resistant and three captan-resistant isolates retested after more than a year in culture displayed the same levels of resistance as previously determined.

DISCUSSION

Resistance to benomyl and other systemic fungicides has become very widespread since the first introduction of systemics in the late 1960s. Development of general resistance is, in part, the result of selection pressure exerted on the pathogens by the slow reduction in concentration of systemics in plant tissue to sublethal amounts by diffusion in the plant tissue and by normal fungicide degradation; it is also the result of the tendency to recommend the least amount of fungicide possible.

The mode of action of systemic fungicides is more specific than that of nonsystemics, usually affecting a single site of action at the cellular level (2). As a result, development of biotypes resistant to the fungicide depends on relatively minor changes in the fungal genome. Greater changes in the genome are required for selection by contact fungicides with their more general mode of action. For this reason, development of resistance to contact fungicides in the field has been a relatively rare occurrence. Ogawa et al (3) list only 17 of 62 contact organic fungicides in general use where resistance has developed, even though many of these have been available for more than 50 yr. In contrast, a wide variety of organisms had developed resistance to nine of the 10 systemics in general use in 1977, even though the first systemic, benomyl, had come into use less than 10 yr before.

The appearance of benomyl-resistant strains of *B. cinerea* was not unusual, but the discovery of captan-resistant strains was unexpected. As benomyl resistance has become more widespread, it has been the practice to control *B. cinerea* by the application of captan-benomyl sprays. With the advent of strains resistant to captan and to captan plus benomyl, this method of control may prove unreliable, although resistance in vitro does not necessarily indicate resistance in vivo.

The observed level of resistance to captan does not adequately explain the erratic control of *B. cinerea* by captan sprays. The incidence of natural captan-resistant biotypes is low, and only the few strains resistant to 1,000 ppm would likely withstand the concentration of fungicide resulting from recommended application rates. The ability of *B. cinerea* isolates to grow over a captan-containing medium without penetration may explain some control failures. Unless coverage of the plant was complete, the mycelium of *B. cinerea* would be capable of growing over the captan with occasional penetrations where no fungicide was deposited.

Crop losses to *B. cinerea*, particularly in years with rain during the flowering season, can be very severe. The development of benomyl- and captan-resistant strains of *B. cinerea* could pose a serious threat to the horticultural industry.

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