Resistance of *Botrytis cinerea* to Benomyl and Iprodione in Vineyards and Greenhouses After Exposure to the Fungicides Alone or Mixed with Captan

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ABSTRACT

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Benomyl-resistant *Botrytis cinerea* was found in southern Ontario vineyards after seven or fewer applications of benomyl. *B. cinerea* with low-level resistance to iprodione (EC_{50} = 2–4 mg/L) was detected in vineyards after 10 or more applications of iprodione or vinclozolin over 4 yr. Iprodione resistance was detected in greenhouses after use of iprodione for 5 yr. In vineyards, captan used in mixtures with either benomyl or iprodione for 1 yr gave protection against Botrytis bunch rot of grapes, but it did not prevent an increase in the incidence of *B. cinerea* resistant to benomyl or iprodione, respectively.

Additional key words: benzimidazole, bunch rot, dicarboximide, gray mold, *Vitis vinifera*

Populations of *Botrytis cinerea* Pers. resistant to benzimidazole and dicarboximide fungicides developed rapidly after their commercial introduction (2,5). Several mathematical models have been advanced indicating that the mixture of two fungicides with different modes of action should delay the development of resistance to either fungicide under most practical conditions (6,10,13,21). However, few experimental data have been presented on which to develop a fungicide use strategy for *B. cinerea*. Our work examined the extent of benomyl and iprodione resistance in populations of *B. cinerea* within Ontario vineyards and greenhouses in relation to the frequency of use of these fungicides. It also examined the efficacy of these fungicides when used as mixtures with captan for controlling *B. cinerea* and for limiting the incidence of resistance to benomyl and iprodione. Resistance of *B. cinerea* to iprodione in Ontario has been reported (16).

MATERIALS AND METHODS

Sensitivity of isolates to benomyl and iprodione. Benomyl-resistant isolates of *B. cinerea* were identified by their growth and sporulation on potato-dextrose agar (PDA, Difco) amended with benomyl at 10 mg/L (for 1979) or at 1 mg/L (for 1980–1983). A water suspension of benomyl (Benlate 50WP) was incorporated into sterile, molten PDA at 50 C and immediately dispensed as 20-ml aliquots into 100-mm-diameter polystyrene petri dishes.

The mycelial growth responses toward iprodione of five monoconidial cultures of *B. cinerea* from Ontario, Canada, were compared with those of cultures with low-level resistance to iprodione (EC_{50} = 2–4 mg/L) imported from Italy (To RV 4) (7) and Greece (A-3) (18). This served to establish a discriminatory concentration to test for the incidence of low-level resistance among Ontario isolates. Dishes were poured with PDA (pH 5.6) amended when molten at 50 C with a water suspension of iprodione (Rovral 50WP) to give 11 concentrations of iprodione from 0.1 to 10 mg/L; iprodione is stable in acidic culture media (May and Baker Canada Inc., *personal communication*). Each dish was centrally inoculated with a 4-mm mycelial plug from the margin of a 3-day culture grown on unamended PDA at 20 C in darkness. Three replicates of inoculated dishes were used per treatment.

After incubation for 3 days at 20 C in darkness, the mean radial growths of colonies were determined from two measurements made at right angles. Mean radial growth of colonies at each concentration of iprodione was expressed as a percentage of the mean radial growth on unamended PDA and subtracted from 100 to give percent inhibition. The linear regression of percent inhibition (nontransformed) with iprodione concentration ($\log_{10}$ transformation) was examined for each replicate series of each culture. The replicate values for a and b in the regression equation [$Y = a + b$ (log X)] and the derived concentrations of iprodione (X) for 50% (EC_{50}) and 90% (EC_{90}) mycelial inhibition (Y) were examined by analysis of variance. Differences between means were evaluated using the Student-Newman-Keuls multiple range test.

A discriminatory concentration of iprodione at 10 mg/L was used in 1980 in a preliminary attempt to identify iprodione-resistant *B. cinerea* in vineyards. For the 1983–1984 surveys and vineyard experiment, however, iprodione at 2 mg/L was considered more appropriate on the basis of our prior characterization of local sensitive and imported cultures with low-level resistance to iprodione.

Vineyard and greenhouse surveys. In September and October 1979, nine vineyards planted mainly with *Vitis vinifera* L. 'Chardonnay,' 'Johannisberg Riesling,' and 'Pinot Noir' ('Gamay Beaujolais') were surveyed, and 12 bunches severely affected by Botrytis bunch rot were sampled from each vineyard. Cultures of *B. cinerea* were obtained by transferring a few macroconidia from each bunch onto PDA. These were illuminated for 10–14 days to induce sporulation for identification. Mycelial subcultures were made onto PDA amended with benomyl at 10 mg/L to test for benomyl resistance. The number of applications of benomyl made during 1977–1979 was recorded for each location.

A second vineyard survey was conducted in September and October 1983; 25 diseased bunches were collected from each of 11 vineyards planted primarily with *V. vinifera* cultivars, and the numbers of commercial or experimental applications of iprodione or vinclozolin (Ronilan 50WP) made from 1980 to 1983 were noted. Cultures obtained in the manner described earlier were incubated for 3 days at 20 C on fungicide-amended PDA and examined for radial growth indicating resistance to either benomyl at 1 mg/L or iprodione at 2 mg/L.

The greenhouse survey was conducted between September 1983 and February 1984. Diseased plant material infected by *B. cinerea* was collected from 16 plant species from 15 greenhouses representing a range of frequency of iprodione use. Plant species included vegetables and floricultural crops (bedding plants, cut and potted flowers, and foliage plants). Samples were incubated in plastic bags in an illuminated growth room, and a small cluster of sporulating conidiophores was transferred to PDA. For each greenhouse

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location, at least 15 isolates were tested for resistance to benomyl and iprodione by the 3-day mycelial growth test described earlier.

Vineyard fungicide experiments. Four experiments (A–D) were conducted in 1980 on cultivars of *V. vinifera* to determine the relative efficacies of iprodione, vinclozolin, and tank mixtures of separately formulated benomyl or iprodione and captan (Captan 50WP) for the prevention of Botrytis bunch rot. The effects of fungicide programs of five applications on the incidence of Botrytis bunch rot and of fungicide resistance were studied.

In experiment A, the fungicides were applied with a Berthoud Vector 1500 airblast sprayer delivering 680 L/ha with a groundspeed of 4.5 km/hr to nonreplicated blocks (each 0.2 ha) of 8-yr-old Johannisberg Riesling vines planted at a spacing of 1.8 × 2.6 m. Three other experiments were conducted on Chardonnay (experiments B and D) and Pinot Noir (experiment C), using randomized complete block designs of three blocks and single-row plots of 10–12 vines spaced at 1.9 × 2.9 m. Fungicides were applied with an over-row hooded-boom hydraulic sprayer driven at 3.3 km/hr, delivering 1,500 L/ha at a line pressure of 2,100 kPa. The rates of fungicides used were benomyl at 0.7 kg/ha, iprodione and vinclozolin at 0.75 kg/ha, and captan at 2.8 kg/ha. The application dates appear as a footnote to Table 1.

Two days before harvest (late September or early October), 150 bunches per plot in experiments B–D were examined on the vine for Botrytis bunch rot. In experiment A, three samples of 100 bunches each were examined in each of the 0.2-ha blocks. To determine the incidence of fungicide resistance, 44 *Botrytis*-infected bunches were collected 2 days before harvest at each location from each of the unsprayed and benomyl/captan-treated plots. The 22 bunches from each of the iprodione and vinclozolin plots were combined as a single sample because of the likelihood that resistant isolates would be cross-resistant to both fungicides (12). From each sample, 38–44 confirmed cultures of *B. cinerea* were tested for resistance to benomyl at 1 mg/L and iprodione at 10 mg/L.

Experiment E was conducted in 1983; part of it was reported previously (17). It compared the efficacies against Botrytis bunch rot of tank mixtures of captan plus iprodione with those of single fungicides in various programs (Table 2). The experiment was conducted in a vineyard that had received 10 applications of iprodione or vinclozolin made to small plots in 1980 and 1981 and four overall commercial applications of iprodione in 1982. The design was a randomized complete block (with three blocks) with single-row plots of 10 7-yr-old Chardonnay vines. Captan (1.4–2.8 kg a.i./ha) and iprodione (0.38–0.75 kg/ha) were applied with the hydraulic sprayer described previously. Programs consisted of three early-season applications (timing 1–3) or three late-season applications (timing 4–6) or full programs of both early- and late-season applications (timing 1–6). The application dates appear as a footnote to Table 2. Sulfur (9 kg/ha) was applied biweekly to control powdery mildew.

On 25 July 1983, 20 clusters were collected from each replicate plot of the check, captan, and early-season iprodione programs. The clusters were arranged on wire screens in trays enclosed in sealed polyethylene bags and incubated at high humidity for 7 days at 20 C in darkness. Ten isolates of *B. cinerea* were obtained from clusters from each of the check and iprodione-treated plots and were characterized for resistance to iprodione at 2 mg/L. During 27–30 September 1983, 150 bunches per plot were examined on the vine for Botrytis bunch rot with sporulation, and the incidence was expressed as the percentage of bunches infected. Thirty-two infected bunches were removed from each plot and individually enclosed in plastic bags to prevent cross-contamination. An isolation was made from each bunch onto PDA, and cultures confirmed as *B. cinerea* were tested for resistance to iprodione at 2 mg/L.

RESULTS

Survey of benomyl resistance in vineyards, 1979. *B. cinerea* resistant to benomyl at 10 mg/L was identified in five of the nine vineyards sampled in 1979 where there had been seven or fewer applications of benomyl during the previous three seasons. The frequency of benomyl-resistant isolates ranged from 17 to 83%. At one location, benomyl had never been used, but it had been applied eight times since 1976 to a strawberry field 400 m upwind in the direction of the prevailing winds. The association between benomyl resistance and the number of applications of benomyl was not significant ($P > 0.05$) using an adjusted chi-square test for independence (23).

The choice of benomyl concentrations of 1 or 10 mg/L in PDA as discriminatory concentrations for diagnosis of benomyl resistance was based on prior characterization of the responses of cultures to benomyl. The mycelial growth of sensitive cultures was abruptly reduced between 0.01 and 0.1 mg/L with an EC_{50} of 0.04 mg/L. In contrast, the benomyl-resistant cultures were progressively inhibited between 3 and 3,000 mg/L with EC_{50} values between 10 and 100 mg/L, and they showed response curves similar to that illustrated by Bollen and Scholten (3).

Efficacy of benomyl plus captan. In experiments C and D, a mixture of benomyl plus captan significantly ($P = 0.05$) reduced the percentage of infected bunches relative to the check, but it was less effective than iprodione (Table 1). In experiments A and D, benomyl plus captan was not more effective than captan alone, and the inefficacy of benomyl was associated with a substantial increase in the percentage of *B. cinerea* isolates resistant to benomyl between 1979 and 1980 (Table 1). In experiment D, benomyl resistance increased from an undetected level to 85% within one

Table 1. Effects of fungicide programs[w] on prevention of bunch rot and changes in the incidence of benomyl-resistant isolates of *Botrytis cinerea* in three vineyards between 1979 and 1980

Experiment	Fungicide program	Bunches Infected (%)	Isolates resistant to benomyl (%)	
			1979	1980
A	Captan[x]	34 b[y]	17	...[z]
	Benomyl + captan	25 b	17	95
	Iprodione + captan	8 a	17	...
B	Check	88 d	17	7
	Iprodione	10 c	17	16
	Vinclozolin	24 c	17	
C	Check	89 g	20	45
	Benomyl + captan	67 f	20	100
	Iprodione	27 e	20	42
	Vinclozolin	21 e	20	
D	Check	47 j	0	54
	Captan	15 i	0	...
	Benomyl + captan	10 i	0	85
	Iprodione	2 h	0	51
	Vinclozolin	8 i	0	

[w] Application dates in 1980: experiment A = 26 June (prebloom), 5 July (postbloom), 25 July, and 14 and 28 August; experiment B = 27 June (prebloom), 8 July (postbloom), 23 July, and 1 and 19 August; experiment C = 17 June (prebloom), 14 July (postbloom), 21 and 31 July, and 18 August; experiment D = 2 July (bloom), 14 July (postbloom), 25 July, and 5 and 15 August.

[x] Fungicide rates: benomyl at 0.7 kg/ha, captan at 2.8 kg/ha, iprodione at 0.75 kg/ha, and vinclozolin at 0.75 kg/ha.

[y] Means in the same experiment followed by a different letter differ significantly ($P = 0.05$) by LSD method.

[z] Data not available.

calendar year after seven applications of benomyl, five of which were a mixture with a full rate of captan (2.8 kg/ha). In the adjacent check and iprodione- or vinclozolin-treated plots, 51–54% of the *B. cinerea* populations were resistant to benomyl (Table 1). None of the isolates was resistant to iprodione at 10 mg/L.

Responses of cultures to iprodione. The linear (radial) mycelial growth rates on PDA of iprodione-sensitive cultures of *B. cinerea* from Ontario were 13.4–13.7 (mean 13.5) mm/day. This contrasted with the lower growth rates of iprodione-resistant cultures from Ontario of 9.8–12.5 (mean 11.2) mm/day, and from Italy and Greece of 10.6 and 11.8 (mean 11.2) mm/day, respectively. The mean mycelial growth rates of cultures with low-level resistance to iprodione were equivalent to 83% of that of the iprodione-sensitive cultures.

The mycelial inhibition of iprodione-sensitive cultures commenced at about 0.1 mg/L of iprodione and was complete at 1.0 mg/L (Fig. 1). In contrast, the linear mycelial growth of cultures with low-level iprodione resistance increased 5-24% at 1.0 mg/L relative to that on unamended PDA, but it was inhibited completely at 10 mg/L. The response patterns of cultures from Ontario and Europe were similar. Linear equations fitted the data well with correlation coefficients (r = 0.86–0.92, 5–9 df) that were highly significant (P = 0.01). The calculated concentrations of iprodione that reduced mycelial growth by 50% (EC_{50}) for resistant isolates from Ontario were 2.43 and 3.55 mg/L. Values for the European cultures were 2.07 and 2.96 mg/L (Table 3). These concentrations were 14–36 times greater than those for the iprodione-sensitive isolates from Ontario (0.10–0.17 mg/L). The EC_{90} values for resistant cultures fell within a narrow range of 6.26–7.47 mg/L and were nine to 14 times greater than those for sensitive cultures (Table 3). The regression coefficients (b) were significantly (P = 0.01) lower and the intercepts on the Y axis (a) were significantly (P = 0.01) higher for the iprodione-sensitive cultures than for the iprodione-resistant cultures (Table 3).

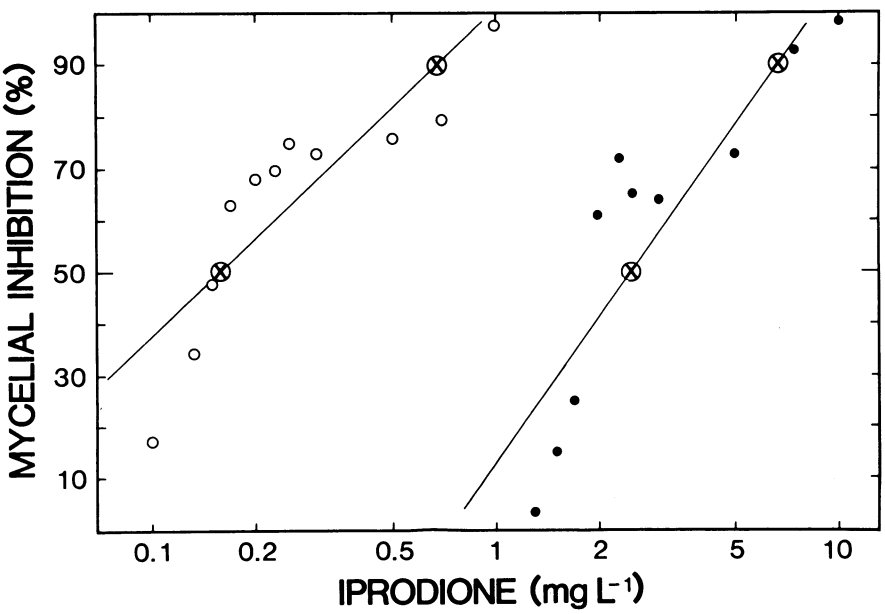

Fig. 1. Inhibition of mycelial growth of Ontario isolates of *Botrytis cinerea* sensitive (S-2, o) and resistant (R-2, •) to iprodione. Each data point corresponds to the mean of three replicates. The linear regression equations for the fitted lines are: S-2, $Y = 100.9 + 64.1 (\log X)$; R-2, $Y = 13.5 + 94.3 (\log X)$. The calculated EC_{50} and EC_{90} points for each isolate are indicated as ⊗.

A discriminatory concentration of iprodione at 2 mg/L in PDA was chosen for the diagnostic test for low-level resistance to iprodione. When mycelial growth on this medium was less than 20% of the growth on unamended PDA, the culture was considered iprodione-sensitive; when growth exceeded 20% on iprodione at 2 mg/L but was less than 20% at 10 mg/L relative to that on PDA, the culture was categorized as having low-level resistance to iprodione. Cultures that were inhibited less than 20% at 10 mg/L were considered highly resistant to iprodione (15).

Surveys of vineyards and greenhouses, 1983. *B. cinerea* with low-level resistance to iprodione was identified in five of the 11 vineyards surveyed in 1983 (Table 4). Each of the vineyards with resistant *B. cinerea* had received at least 10 applications of iprodione or vinclozolin either as commercial applications or as small-plot experimental treatments. The development of iprodione resistance was related to the use of 10 or more applications of iprodione or vinclozolin (adjusted chi-square = 2.753; $P > 0.05$) (23). Benomyl-resistant *B. cinerea* was isolated from each of the 11 vineyards, where it accounted for between 19 and 88% of the population (mean of 48%).

Ten of the 15 greenhouses examined had *B. cinerea* with low-level resistance to iprodione, and in nine locations, this was associated with multiple applications of iprodione either as a regular program (treatment every 7–10 days) or on an occasional basis (treatment less than once per month) over a period of 5 yr (Table 5). Iprodione-resistant *B. cinerea* was found

Table 2. Effects of programs of iprodione (I) and captan (C) used singly or as mixtures (C-I) at full rates or half rates on the incidence of Botrytis bunch rot of grapes, the percentage of iprodione-resistant isolates of *Botrytis cinerea* from bunches, and the incidence of bunches infected by iprodione-resistant *B. cinerea*

Fungicide applications (timing)[x]			Bunches with Botrytis bunch rot	*B. cinerea* isolates resistant to iprodione	Bunches infected by iprodione-resistant *B. cinerea*
1-3	4-6	Rate[y]	(%)	(%)	(%)
I	...	F	56 e[z]	55 b	31 cd
...	I	F	44 d	81 c	36 d
I	I	F	38 bcd	97 d	37 d
C	I	F	38 bcd	95 d	37 d
C	C	F	41 cd	8 a	3 a
C-I	C-I	F	22 a	74 c	16 b
C-I	C-I	H	31 abc	88 cd	27 c
Unsprayed check	Unsprayed check	...	81 f	5 a	5 a

[x] Application times in 1983: 1 = 29 June (bloom), 2 = 7 July (postbloom), 3 = 15 July, 4 = 25 July, 5 = 8 August, and 6 = 23 August.
[y] F = full rate: captan at 2.8 kg/ha and iprodione at 0.75 kg/ha. H = half rate: captan at 1.4 kg/ha and iprodione at 0.38 kg/ha.
[z] Means in the same column followed by a different letter differ significantly (P = 0.05) according to Duncan's multiple range test.

Table 3. The *Y* intercept (*a*) and regression coefficient (*b*) for the linear regression[y] of the inhibition of mycelial growth (*Y*) of seven isolates of *Botrytis cinerea* against $\log_{10}$ of the concentration of iprodione (*X*) and the computed concentrations for 50% (EC_{50}) and 90% (EC_{90}) inhibition of linear mycelial growth

Designation and source of isolate	*Y* intercept (*a*)	Regression coefficient (*b*)	Concentration of iprodione for 50 and 90% mycelial inhibition (mg/L)	
			EC_{50}	EC_{90}
Iprodione-sensitive isolates from Ontario				
GSS	105.7 f[z]	56.4 a	0.10 a	0.53 a
S1	101.8 ef	66.8 b	0.17 a	0.67 b
S2	100.9 e	64.0 b	0.16 a	0.68 b
Iprodione-resistant isolates from Ontario				
R2	13.8 c	93.9 d	2.43 c	6.47 d
R1	−18.2 a	123.9 e	3.55 e	7.47 e
Iprodione-resistant isolates from Europe				
To RV 4 (Italy)	23.8 d	83.2 c	2.07 b	6.26 c
A-3 (Greece)	2.8 b	100.0 d	2.96 d	7.43 e

[y] Equation for linear regression: $Y = a + b(\log X)$, where *Y* is mycelial inhibition (%) and *X* is concentration of iprodione (mg/L) incorporated in PDA.
[z] Means in the same column followed by different letters differ significantly ($P = 0.01$) according to the Student-Newman-Keuls multiple range test.

in two greenhouses on stock very recently imported from the United States, and in one of these greenhouses, iprodione had not been used. In four other locations where iprodione had never been used, iprodione resistance was not detected. The association between the use of iprodione and the presence of iprodione-resistant *B. cinerea* was significant ($P = 0.05$) using an adjusted chi-square test for independence (23). Benomyl-resistant isolates were found in 14 of the 15 greenhouses with incidences of 71–100% (mean of 87%).

Efficacy of iprodione and iprodione/captan programs. On 25 July 1983, 92% of the clusters from check plots were infected by *B. cinerea*, but none of the 30 isolates tested was resistant to iprodione. In contrast, 63% of clusters treated with iprodione were infected, and several isolates from each replicate plot were resistant to iprodione (mean of 47%). Only 25% of the captan-treated clusters were infected, but this incidence was not significantly different ($P > 0.05$) from that in the plots treated with iprodione.

By late September, full programs of six applications of iprodione or captan used separately were only moderately effective in reducing the incidence of Botrytis bunch rot to 38–41% from 81% in the check (Table 2). The late-season use (timing 4–6) of iprodione, with or without the early-season use (timing 1–3) of captan, also was moderately effective, but the sole use of an early-season iprodione program was significantly worse. The best protection was provided by the program of six applications of a mixture of full rates of captan and iprodione, which reduced bunch rot to 22%.

In September, the incidences of *B. cinerea* resistant to iprodione were 5 and 8% in the check and captan-treated plots, respectively (Table 2). Incidence was significantly greater (55%) after three early-season applications (1–3) of iprodione, and the incidence increased further to 81–97% where three late-season applications (4–6) or the complete program of six applications (1–6) of iprodione had been used. The six-spray programs of mixtures of iprodione and captan applied at full rates (F) and half rates (H) resulted in 74 and 88% resistance, respectively. These values were not significantly different ($P > 0.05$) from each other, but the incidence in the full-rates mixture program (74%) was significantly ($P = 0.05$) lower than that in the complete six-spray program of iprodione alone (97%).

Table 4. Frequency of *Botrytis cinerea* with low-level resistance to iprodione in Niagara region vineyards in relation[a] to numbers of applications of iprodione and vinclozolin during 1980–1983

Location (vineyard)	Iprodione-resistant isolates (%)	Applications of iprodione and vinclozolin
1	71	20
2	36	10
3	24	13
4	16	16
5	13	10
6	0	16
7	0	10
8	0	7
9	0	4
10	0	2
11	0	2

[a] The association between iprodione-resistant *B. cinerea* in vineyards and 10 or more applications of iprodione or vinclozolin: chi-square adjusted for continuity = 2.753 ($P > 0.05$).

The incidence of bunches infected by iprodione-resistant *B. cinerea* was obtained by multiplying the individual replicate data of the percentage of bunches with Botrytis bunch rot by the mean incidence of isolates resistant to iprodione for the respective treatment (Table 2). In the captan and check plots, 3 and 5%, respectively, of bunches were infected by iprodione-resistant *B. cinerea*. In plots treated with iprodione alone, incidence was 31–37%, but it was significantly ($P = 0.05$) less (16%) where a mixture of full rates (F) of captan and iprodione had been used. The half-rates program (H) was less effective than the full-rates program but was marginally better than programs involving the late season use of iprodione alone.

Table 5. Frequency of *Botrytis cinerea* with low-level resistance to iprodione in greenhouse crops in relation[a] to the use of iprodione

Location (greenhouse)	Iprodione-resistant isolates (%)	Use of iprodione[b]
1	96	Regular
2	48[c]	Regular
3	35	Regular
4	33	Occasional
5	19	Occasional
6	16	Regular
7	8	Occasional
8	6[c]	Never
9	5	Occasional
10	3	Occasional
11	0	Occasional
12	0	Never
13	0	Never
14	0	Never
15	0	Never

[a] The association between the use of iprodione and the presence of iprodione-resistant *B. cinerea* was significant ($P = 0.05$) using an adjusted chi-square test for independence.
[b] Regular use = treatment every 7–10 days; occasional use = treatment less than once per month during previous 5 yr.
[c] Resistant isolates from newly imported stock.

DISCUSSION

The development of benomyl resistance within populations of *B. cinerea* in Ontario vineyards occurred after seven or fewer applications of benomyl, comparable to earlier observations in Europe (3,20). The widespread distribution of benzimidazole resistance in Ontario vineyards and greenhouses has not been described previously. Its occurrence in a vineyard not treated with benzimidazole fungicides parallels observations in Swiss vineyards (20) and was explained either

by the prior existence of resistant populations (4) or more probably by the spread of airborne resistant inoculum from neighboring treated areas (19,20). The development of benomyl resistance in the check and iprodione-treated plots of experiment D was attributed to the spread of resistant inoculum from the benomyl/captan-treated plots. The reduced efficacy of benzimidazole fungicides resulted in a reliance on the more recently introduced dicarboximide fungicide, iprodione.

Low-level resistance to iprodione in populations of *B. cinerea* in Ontario was first observed in the 1983 experiment in a vineyard treated during 1980–1982 with 14 applications of iprodione or vinclozolin. Resistant isolates were recovered from plots treated with three applications of iprodione before 25 July, whereas none of the 30 isolates from the check plots was resistant. The detection of iprodione-resistant isolates in the check and captan plots in late September was attributed to the wind dispersal of resistant inoculum from neighboring iprodione-treated plots and to the greater chance of detecting resistance with the greater number of bunches sampled.

The emergence of low-level resistance to iprodione in Ontario after 5 yr of use in greenhouses or a minimum of 10 applications in some vineyards follows the pattern observed in other countries where dicarboximide fungicides were registered for commercial use earlier than in North America (1,7,11,18). To our knowledge, this contribution and the brief preliminary report (16) constitute the first documented occurrence in North America of *B. cinerea* with low-level resistance to iprodione.

The EC_{50} concentrations of iprodione of 2–4 mg/L for the 50% inhibition of linear mycelial growth of cultures of *B. cinerea* from Ontario with low-level iprodione resistance resembled those reported from New Zealand (1) and Europe (7,11,18). The resistance factors, defined as the EC_{50} value of the resistant isolate divided by that of the sensitive isolate (12), were 14–36 for the cultures from Ontario with low-level resistance and resembled those in earlier reports (7,11,12,18). These values contrasted with EC_{50} values of iprodione in excess of 100 mg/L for highly resistant isolates obtained in vitro (15) with resistance factors greater than 625. Cultures from Ontario with low-level resistance to iprodione produced macroconidia and sclerotia abundantly, but the linear mycelial growth rate was slightly less than that of sensitive isolates. In these characteristics, they further resembled European isolates (7,8,11,18) and differed from the slower-growing scleroconidial cultures selected in vitro that had a high level of iprodione resistance (15).

The use of mixtures of a multisite fungicide (e.g., captan) with a specific-site fungicide (e.g., benomyl or iprodione) has been widely discussed as a strategy for delaying the development of fungicide resistance (6,10,13,21). Laboratory experiments (22) and field observations (6) support the validity of this approach for some situations. In our 1980 experiment, captan applied with benomyl did not prevent an increase in the incidence of benomyl resistance or its development within one season in experiment D. Likewise, in our 1983 experiment, the mixture of captan and iprodione reduced only slightly the incidence of iprodione-resistant *B. cinerea* compared with the iprodione program in a vineyard with a history of dicarboximide treatments. Our results agree with recent findings (8,9,14) relating to dicarboximide fungicides indicating that their mixture with chemically dissimilar fungicides may give satisfactory control of *B. cinerea* but that this strategy did not prevent an increase in the incidence of dicarboximide resistance within the fungal population.

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