Dicarboximide-Resistant Isolates of *Botrytis cinerea* from Table Grape in Chile: Survey and Characterization

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ABSTRACT

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Two to four applications annually of the dicarboximide (DC) fungicides iprodione and vinclozolin have been used widely for 10–15 yr to control gray mold, caused by *Botrytis cinerea*, of grapes in Chile. Control failures attributable to field resistance to DC fungicides have not been reported, although the frequency of low-level resistant (LLR) isolates increased from 2 to 74.9% between the 1987–1988 and 1993–1994 growing seasons; 0.3 and 0.6% of the isolates tested during 1992–1993 and 1993–1994, respectively, were highly resistant based on mycelial growth inhibition on PDA amended with 10 mg/L of vinclozolin. The EC_{50} for mycelial growth varied from 2.51 to 9.02 and from 2.00 to 18.16 mg/L of vinclozolin among isolates from commercial plantations during 1992–1993 and 1993–1994, respectively. The resistant factor (RF) for the most resistant LLR isolate was 60.13, although most LLR isolates had RF values of <30. Cross-resistance among DC fungicides and to dicloran and PCNB was demonstrated. Although resistant isolates lost some fitness attributes, e.g., higher osmolarity sensitivity, they were virulent and equally inhibited when inoculated nectarine fruit were treated with commercial rates of iprodione, procymidone, and vinclozolin. Conidial germination and mycelial growth of highly sensitive (HS) isolates (EC_{50} for mycelial growth = ≤0.5 mg/L) collected from grapevines never exposed to DC fungicides were completely inhibited by 10 mg/L of iprodione or vinclozolin. Conidial germination of LLR isolates (EC_{50} for mycelial growth = 2–5 mg/L) was inhibited by only 0.7–9.7%, whereas mycelial growth was arrested by 89.0–91.6%. Inhibition of growth of LLR isolates by iprodione or vinclozolin may partially explain the relatively high degree of control of gray mold of table grape that is still possible with DC fungicides after 10–15 yr of continuous use.

Gray mold, caused by *Botrytis cinerea* Pers.:Fr., is the most destructive disease of table grapes (*Vitis vinifera* L.) in Chile. Disease incidence and severity are dependent on the microclimatic conditions that develop under the high-trellis system. This trellis system is the most popular system used for table grape production in Chile and is characterized by a 2-m high horizontal plane of vegetation. High disease incidence and severity occur when wet and cool conditions persist during the growing season, particularly if they are prevalent near harvest (4,14,15,24).

An integrated control program, including both canopy management and fungicide treatments, has been suggested for the past 5 yr to improve the control of gray mold while lowering the traditional number of fungicide applications. Fungicide regimes currently used include two to four dicarboximide (DC) sprays of either iprodione or vinclozolin (14,15,29). These fungicides have been used widely during the past 10–15 yr in most vineyards in the Central Valley of Chile, where conditions are often favorable for disease development. Although the risk of *B. cinerea* developing resistance to DC fungicides is considered high, there are as yet no reports of major control failure under vineyard conditions in Chile.

Low-level resistant (LLR) and highly resistant (HR) isolates of *B. cinerea* to DC fungicides have been described. The effective concentration for a 50% reduction of mycelial growth (EC_{50}) is 2–10 mg/L for LLR isolates, >10 mg/L for HR isolates, <2 mg/L for sensitive (S) isolates, and ≤0.5 mg/L for highly sensitive (HS) isolates. The mycelial growth inhibition (MGI) on 10 mg/L of a DC fungicide (MGI_{10}) is >95% for S isolates, 51–95% for LLR isolates, and <50% for HR isolates (1,3,7,10). Dicarboximide resistance is associated with high osmotic sensitivity and results from mutation of alleles of the gene for sensitivity, *Daf1*; alleles *Daf1*LR and *Daf1*HR are responsible for low and high levels of resistance, respectively (7).

Growers are concerned about the likelihood of the appearance, increase, and rapid dissemination of resistant isolates among vineyards. This could result in partial or complete loss of disease control with DC fungicides, as has been reported for outdoor (2,3,10,16,18,22,25) and protected crops (12,18,27,32,33). However, only LLR isolates of *B. cinerea* have been reported in Chile (5,29). The objectives of this study were to survey for resistance to DC fungicides in the Central Valley of Chile, to characterize LLR isolates, and to study the effect of DC fungicides on LLR isolates of *B. cinerea*.

MATERIALS AND METHODS

Fungicides. The fungicides used in this study were iprodione (Rovral 50WP), procymidone (Sumisclex 50DF), vinclozolin (Ronilan 50WP), dicloran (Botran 75WP), and PCNB (quintozene; Brassicol 20WP). For in vitro studies, an aqueous suspension of each fungicide was prepared and added aseptically to molten (50 C) sterile potato-dextrose agar acidified with 0.5 ml/L of 1 N lactic acid (PDA). Unless stated otherwise, rates are given as active ingredient.

Effect of dicarboximides on conidial germination and mycelial growth. Twenty-eight isolates of *B. cinerea* varying in EC_{50} values from 0.15 to 6.5 mg/L of vinclozolin were collected from commercial vineyards, and three HS isolates (EC_{50} <0.5 mg/L) were obtained

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from backyard grapevines never sprayed with DC fungicides. All isolates were tested for conidial germination on 2% water agar (WA) and WA amended with 10 mg/L of iprodione (IWA) or vinclozolin (VWA). Conidia were produced on PDA incubated for 10–15 days at 23 C before they were harvested by washing the surface of the cultures with sterile water (SW) containing 0.01% Tween 20. A hemacytometer was used to adjust the concentration of conidia to 1×10^6 per milliliter, then 0.2 ml was aseptically spread in triplicate onto WA, IWA, and VWA culture dishes. After 18 hr of incubation in the dark at 23 C, germination was determined by observing at least 200 conidia per isolate under the light microscope. Only a conidium with a germ tube at least twice its length was considered germinated. This experiment was repeated twice.

Using the procedure described above, conidia from one HS and four LLR isolates were spread in triplicate on WA and WA amended with iprodione or vinclozolin at 10, 20, 40, and 60 mg/L. Conidia were incubated for 18 h at 23 C before germination was determined, then small plugs of agar medium (0.2 cm^2), with approximately 600 germinated conidia, were aseptically transferred to PDA containing either iprodione or vinclozolin at each of the concentrations listed above. Culture dishes were incubated for 4 days at 23 C before determining MGI relative to unamended PDA. The experiment was repeated twice.

During the 1992–1993 growing season, the sensitivity of the population of *B. cinerea* found in two vineyards (Colina and Doñihue) of cv. Thompson Seedless was determined on the basis of conidial germination tests and MGI. For each vineyard, a composite sample of conidia was obtained from 40 sporulating berries selected individually from 40 clusters, collected from at least 10 plants from each of three replicate plots. Conidia were washed in SW containing 0.01% Tween 20, and 0.2-ml aliquots of 1×10^6 conidia per milliliter were seeded onto WA, IWA, or VWA. Four dishes of each culture medium were incubated at 23 C for 18 hr before germination was determined for at least 2,000 conidia for each of three replicates. Twelve selected isolates from each of three replicates were obtained by transferring germinated conidia to PDA. The isolates were incubated for 4 days at 23 C, and then agar plugs were transferred to PDA amended with 10 mg/L of either iprodione or vinclozolin. Culture dishes were incubated for 4 days at 23 C before the MGI was determined. Data were analyzed by analysis of variance using a completely randomized design; means were separated according to the Waller-Duncan *k*-ratio *t* test (31).

Effect of dicarboximide-free period. The effect on pathogen sensitivity of withholding dicarboximide sprays was determined in a commercial cv. Thompson Seedless vineyard. The following DC spray programs were maintained in 0.16-ha plots: 1) four sprays (750 g/ha) of vinclozolin per season from 1990–1991 to 1992–1993, 2) four sprays of vinclozolin per season in 1990–1991 and 1991–1992 but no DC fungicides in 1992–1993, and 3) four sprays of vinclozolin in 1990–1991 but no DC fungicides in 1991–1992 and 1992–1993. The experimental design was a completely randomized block with three replicates. Conidia of *B. cinerea* were obtained from 10 sporulating berries collected from at least 10 plants per replicate. Conidial germination was determined as described above on WA, IWA, and VWA. Three isolates were then selected from each of the three replicate dishes per treatment on WA transferred to PDA, and the resulting colonies were tested to determine MGI_{10} values as described above. Data were subjected to analysis of variance, and means were separated according to the Waller-Duncan *k*-ratio *t* test (31).

Survey of fungicide resistance. From the 1987–1988 to the 1993–1994 growing season, isolates of *B. cinerea* were obtained from table grapes, primarily cvs. Thompson Seedless and Flame Seedless, from vineyards located about 220 km from north to south in the Central Valley of Chile (32–33° south). All vineyards had a history of use of DC fungicides. For comparison, 13 additional isolates were obtained from grapes growing in backyards at least 10 km from the nearest commercial vineyard and with no known history of exposure to DC fungicides. Isolates were obtained from two or three berries with symptoms of gray mold per vineyard from 1987–1988 to 1992–1993; 10 berries were collected per sampling site in 1993–1994. Collections were made before harvest (February and March) each season. Each berry was placed in a small plastic glass (5 cm in diameter) and transported in a cooler to the laboratory. Single conidiophores of *B. cinerea* were selected

Table 1. Effects of iprodione and vinclozolin on conidial germination of isolates of *Botrytis cinerea* with various degrees of mycelial sensitivity to vinclozolin

Degree of sensitivity[x]	EC_{50}[y] (mg/L)	No. of isolates	Percent inhibition of conidial germination[z]	
			Iprodione (10 mg/L)	Vinclozolin (10 mg/L)
Highly sensitive	0.1–0.5	3	100.0	100.0
Sensitive	0.6–2.0	18	15.4	17.5
Low-level resistant	2.1–5.0	8	2.6	2.7
	5.1–6.5	2	3.5	0.5

[x] Categories after Beever and Brien (1), Beever et al (3), and Faretra and Pollastro (7). Highly sensitive isolates were collected from grapevines with no history of dicarboximide fungicide use, and sensitive isolates were collected from commercial vineyards with a known history of such fungicide use.

[y] Values for inhibition of mycelial growth on potato-dextrose agar amended with vinclozolin.

[z] At least 600 conidia per isolate were observed per each experiment after 18 hr of incubation at 23 C on water agar amended with either iprodione or vinclozolin.

Table 2. Degree of mycelial growth inhibition for sensitive and low-level resistant isolates of *Botrytis cinerea* after subculture of germinated conidia in the presence of dicarboximide fungicides[z]

Fungicide (mg/L)	Percent inhibition			
	Sensitive		Resistant	
	Conidial germination	Mycelial growth	Conidial germination	Mycelial growth
Iprodione				
0	0.0	0.0	0.0	0.0
10	100.0	100.0	9.7	91.6
20	100.0	100.0	6.7	92.8
40	100.0	100.0	13.0	92.8
60	100.0	100.0	8.7	93.8
Vinclozolin				
0	0.0	0.0	0.0	0.0
10	100.0	100.0	0.7	89.0
20	100.0	100.0	2.2	91.5
40	100.0	100.0	11.0	92.8
60	100.0	100.0	11.0	92.3

[z] Percent inhibition was determined for one highly sensitive isolate (EC_{50} = 0.05 mg/L of vinclozolin) collected from grapevines unexposed to dicarboximide fungicides and from four low-level resistant isolates (EC_{50} = 3–5 mg/L of vinclozolin) collected in commercial vineyards with a history of such fungicide use. Condial germination was tested on 2% water agar, and mycelial growth inhibition was determined on potato-dextrose agar.

under a dissecting microscope, placed on PDA, and incubated at 23 C for 48 to 72 hr. Subcultures were obtained from hyphal tips growing on PDA to test sensitivity. Isolates were maintained on PDA at 5 C prior to testing.

The mycelial growth sensitivity of each isolate to DC fungicides was determined on PDA amended with 10 mg/L of vinclozolin (VPDA). Plugs (5 mm in diameter) taken from actively growing mycelium on PDA were placed in the centers of each of three dishes containing VPDA and on three nonamended PDA dishes. Culture dishes were incubated for 4 days at 23 C until nonamended dishes were completely covered by mycelium, then the MGI was determined. Isolates with a radial growth of <2.5 mm (MGI_{10} >95%) were considered S, those with a radial growth of 2.5–27.5 mm (MGI_{10} 50–94%) were considered LLR, and those with a radial growth >27.5 mm (MGI_{10} <50%) were considered HR (1,7,10).

Dose-response relationships. The EC_{50} value for vinclozolin was determined for 96, 54, 90, 52, and 10 isolates each growing season from 1989–1990 through 1993–1994, respectively. Agar plugs of each isolate taken from the margins of actively growing colonies in PDA were seeded in triplicate on PDA ammended with 0.1, 0.5, 0.7, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 10.0, 12.0, 15.0, 20.0, and 30.0 mg/L of vinclozolin. The EC_{50} values were estimated by linear regression analysis, where X = log concentration and Y = probit % of mycelial inhibition (28).

Cross-resistance and biological characterization. Six S isolates (EC_{50} = 0.17–0.24 mg/L of vinclozolin) and seven LLR isolates (EC_{50} = 3.7–4.98 mg/L of vinclozolin) were characterized for cross-resistance to iprodione, procymidone, and vinclozolin and to the halogenated hydrocarbon compounds dicloran and PCNB. Tests were performed in triplicate on dishes of PDA amended with 2, 5, or 10 mg/L of each DC fungicide; with 5, 10, 20, or 30 mg/L of dicloran; and with 10, 15, 30, or 50 mg/L of PCNB. Cultures were incubated at 23 C for 4 days before determining radial growth of mycelium. Data were analyzed according to the least squares test (30).

Several comparisons were made for the same *B. cinerea* isolates: 1) Conidial production was determined on nonamended PDA following incubation for 8 days at 23 C under ultraviolet light (310 nm wavelength). Conidia were harvested by washing each of three dishes with 3 ml of distilled water containing 0.01% Tween 20 and were counted with a hemacytometer. 2) The growth rate of each isolate was determined on PDA by measuring the radial growth 48 and 96 hr after incubation at 23 C. 3) The total number of sclerotia produced on 9-cm-diameter dishes containing PDA was determined after 30 days of incubation at 23 C. 4) Osmotic sensitivity was evaluated as radial mycelial growth after 4 days at 23 C on PDA + 0.68 M NaCl, relative to growth on nonamended PDA. Three replicates were used in all of the above tests.

In vivo test. The effectiveness of 250, 500, and 750 mg/L of iprodione, procymidone, and vinclozolin against an S isolate (EC_{50} = 0.17 mg/L of vinclozolin) and an LLR isolate (EC_{50} = 4.98 mg/L of vinclozolin) of *B. cinerea* was tested in vivo on nectarines (*Prunus persica* (L.) Batsch var. *nectarina* (Aiton) Maxim. 'Fantasia'). Fruit were selected for similar maturity (14° Brix), surface-disinfested in 95% ethanol followed by 1% NaOCl for 2 min each, and rinsed in sterile water. Fruit were wounded, immersed in the fungicide suspensions for 2 min, and allowed to dry before being inoculated with 10 µl of a 1×10^4 conidia per milliliter suspension obtained by washing 10- to 15-day-old PDA culture dishes with SW + 0.01% Tween 20. Fruit were incubated in humid chambers for 4 days at 23 C, then the diameters of the resulting lesions were measured. The experiment was a $3 \times 3 \times 2$ factorial (fungicide × rate × isolate) in a completely randomized design with four replicates. Data were analyzed by analysis of variance (30), and mean separation was done with Duncan's multiple range test.

Table 3. Evaluation of the sensitivity to dicarboximide fungicides of *Botrytis cinerea* isolates from two commercial vineyards in Chile, based on inhibition of conidial germination and mycelial growth

	Percent inhibition[y]			
	Iprodione (10 mg/L)		Vinclozolin (10 mg/L)	
Vineyard[x]	Conidial germination	Mycelial growth	Conidial germination	Mycelial growth
Colina	17.6 a[z] (0.0–40.5)	67.4 b (38.2–100.0)	19.5 a (0.0–42.1)	83.9 b (69.7–100.0)
Doñihue	6.9 a (0.0–41.3)	71.7 b (65.7–100.0)	4.7 a (0.0–27.1)	82.6 b (74.5–100.0)

[x] Both vineyards were table grape cv. Thompson Seedless with a history of dicarboximide use in the previous 5 yr.
[y] Percent inhibition of conidial germination was determined for 2,000 conidia per each of three replicates on water agar containing 10 mg/L of either iprodione or vinclozolin. Mycelial growth inhibition was an average of 12 isolates per replicate determined on potato-dextrose agar amended with 10 mg/L of either fungicide. Ranges are given in parentheses.
[z] Means in the same row followed by the same letter are not significantly different according to the Waller-Duncan k-ratio t test ($P < 0.05$).

Table 4. Effect of withholding dicarboximide treatments on the sensitivity of *Botrytis cinerea* populations from table grapes to iprodione and vinclozolin based on inhibition of conidial germination and mycelial growth

	Percent inhibition[y]			
	Iprodione (10 mg/L)		Vinclozolin (10 mg/L)	
Treatment[x]	Conidial germination	Mycelial growth	Conidial germination	Mycelial growth
Full program	42.4 a[z]	91.4 a	33.3 a	95.0 a
Sprays withheld				
1 yr	57.8 a	100.0 b	50.7 b	100.0 a
2 yr	95.5 b	100.0 b	56.7 b	100.0 a

[x] Full program = four applications (750 g/ha) of vinclozolin per year; sprays withheld = no applications of vinclozolin or iprodione for 1 or 2 yr.
[y] Percent inhibition of conidial germination was determined for 500 conidia per each of three replicates on water agar containing 10 mg/L of either iprodione or vinclozolin. Mycelial growth inhibition was an average of three isolates per replicate determined on potato-dextrose agar amended with 10 mg/L of either fungicide.
[z] Means in the same column followed by the same letter are not significantly different according to the Waller Duncan k-ratio t test ($P < 0.05$).

RESULTS

Effect of dicarboximides on conidial germination. Conidial germination of HS isolates with an EC_{50} of ≤0.5 mg/L was completely inhibited on WA containing 10 mg/L of either iprodione or vinclozolin, in comparison to inhibition of only 0.5–3.5% for various LLR isolates (EC_{50} = 2–6.5 mg/L). Sensitive isolates from vineyards treated with DC fungicides (EC_{50} = 1–2 mg/L) were intermediate, exhibiting 15.4 and 17.5% inhibition of conidial germination with iprodione and vinclozolin, respectively (Table 1). Germ tubes that did develop were highly distorted. For example, germ tubes of LLR isolate 93.70.1 (EC_{50} = 8.76 mg/L) were 88 and 248 µm long on IWA and VWA, respectively, after 18 hr of incubation at 23 C, compared with 543 µm for the same isolates on nonamended WA. Mycelial growth of LLR isolates was inhibited by 89.0–93.8% when germinated conidia were subcultured on PDA amended with 10–60 mg/L of

either iprodione or vinclozolin. The highest concentrations used may exceed the solubility of the fungicides in water and may explain a lack of response obtained with vinclozolin above 20 mg/L (Table 2). Similar significant ($P < 0.05$) differences were obtained between the inhibition of conidial germination and mycelial growth for *B. cinerea* isolates obtained from two additional commercial vineyards (Table 3).

Effect of dicarboximide-free period. The conidial germination of isolates collected from grapevines sprayed with DC fungicides four times annually during the previous 3 yr was inhibited by 33.3 and 42.4% by 10 mg/L of vinclozolin and iprodione, respectively. The percent inhibition was significantly greater ($P < 0.05$) for *B. cinerea* isolates obtained from grapevines that had not been treated with DC fungicides for 1 or 2 yr, e.g., 57.8 and 95.5% for iprodione after 1 and 2 yr without DC fungicides, respectively (Table 4). Mycelial growth of isolates from vines sprayed all 3 yr was inhibited by 91.4 and 95.0% by 10 mg/L of iprodione and vinclozolin, respectively; mycelial growth was completely inhibited for isolates obtained from vines untreated with DC fungicides for the previous 1 or 2 yr.

Survey and resistance characterization. The proportion of LLR isolates of *B. cinerea* increased considerably between the 1987–1988 and 1993–1994 growing seasons in the Central Valley of Chile. During 1987–1988, only 2% of the isolates tested were able to sustain mycelial growth on 10 mg/L of vinclozolin, whereas 74.9% of the isolates collected during 1993–1994 had an MGI_{10} between 50 and 95%; 0.6% of the isolates collected during 1993–1994 were considered highly resistant on the basis of their MGI_{10} values (Table 5).

The EC_{50} values for vinclozolin for isolates of *B. cinerea* recovered from grapevines untreated with DC fungicides varied from 0.15 to 0.27 mg/L, while EC_{50} values for isolates from commercial vineyards where DC fungicides had been used at least twice a year ranged from 0.15 to 6.16 mg/L in 1989–1990, from 0.15 to 3.48 mg/L in 1990–1991, from 0.1 to 7.0 mg/L in 1991–1992, from 0.1 to 8.76 mg/L in 1992–1993, and from <0.5 to 18.16 mg/L in 1993–1994.

Isolates of *B. cinerea* were cross-resistant to DC fungicides. For instance, the MGI of seven LLR isolates was similarly inhibited (mean differences were not significant, $P < 0.05$) by 5 mg/L of either iprodione (58%), procymidone (58%), or vinclozolin (65%). In contrast, LLR and S isolates differed significantly ($P < 0.01$) in the degree of MGI provided by each DC fungicide, e.g., 58–65% vs. 99–100%, respectively. Similarly, cross-resistance between DC fungicides and halogenated hydrocarbon fungicides was obtained for the same LLR isolates. For instance, 5 mg/L of dicloran inhibited mycelial growth of LLR and S isolates by 40 and 81%, respectively, and 10 mg/L of PCNB arrested mycelial growth of LLR and S isolates by 78 and 68%, respectively.

Sensitive isolates produced significantly ($P < 0.05$) more sclerotia, showed a slightly higher growth rate, were significantly ($P < 0.05$) less inhibited by 0.68 M NaCl (2.7 vs. 16.4%), and produced slightly fewer conidia than LLR isolates.

In vivo test. Each DC fungicide, at concentrations equivalent to commercial applications, equally arrested the LLR and S isolates of *B. cinerea* when inoculated onto nectarines. No significant differences for percent lesion inhibition were obtained between LLR and S isolates. Regardless of the concentration, differences among fungicides were statistically significant ($P < 0.05$); vinclozolin and iprodione provided the highest control (90.3 and 97.0%, respectively) and procymidone provided the lowest (84.2%). None of the possible interactions among fungicides, concentrations, and isolates was statistically significant ($P < 0.05$).

DISCUSSION

The frequency of *B. cinerea* isolates that grew on agar containing 10 mg/L of vinclozolin increased progressively from 2 to 75.5% between the 1987–1988 and 1993–1994 growing seasons in table grape vineyards treated at least twice a year with a dicarboximide fungicide. However, 74.9% were LLR isolates with EC_{50} values <9.02 mg/L. This level of resistance is above the range previously described in Chile (5) and is similar to the level of resistance reported for *B. cinerea* on grapes and other crops under field conditions elsewhere (1,3,7,8,12, 18,21,29,33).

Isolates of *B. cinerea* from grapevines not previously exposed to DC fungicides were highly sensitive, with EC_{50} values for mycelial growth <0.5 mg/L. Similar isolates were also detected in commercial vineyards with a long history of use of DC fungicides. A resistant factor (RF) of 60.13 was obtained for the most resistant LLR isolate, relative to the most sensitive isolate ($EC_{50} = 0.15$ mg/L), but the majority had RF values below 30. This is somewhat higher than the RF commonly described for low resistant populations of *B. cinerea* (7,8,16,21). Nevertheless, there has been no report of complete failure of gray mold control in any of the vineyards from which these resistant *B. cinerea* isolates were recovered, although a partial loss in efficacy in vineyards with low resistant populations has been reported elsewhere (22).

DC fungicides inhibit conidial germination and arrest the mycelial growth of sensitive isolates of *B. cinerea* (2,3,19, 23,25,26). However, these results show that DC fungicides only partially inhibit or do not inhibit conidial germination in LLR isolates. Germ tubes developed by LLR isolates on WA containing 10 mg/L of either iprodione or vinclozolin were several times the length of the conidium before swelling and bursting occurred as described previously (6,19,25). Only conidia of very sensitive isolates of *B. cinerea* exhibited near 100% inhibition of both conidial germination and mycelial growth in the presence of 10 mg/L of either iprodione or vinclozolin. In contrast, conidial germination increased in the presence of 10 mg/L of vinclozolin as the EC_{50} for mycelial growth increased from 1 to 6.16 mg/L (Table 1). Similar results have been reported previously for *B. cinerea* isolates from grapevines (3).

There appears to be little correlation between the disruption of conidial germination and mycelial growth inhibition for LLR isolates of *B. cinerea*. For instance, conidial germination was inhibited only 0.7–9.7% among LLR isolates exposed to 10 mg/L of iprodione or vinclozolin, while mycelial growth of the same isolates was inhibited by 89.0–91.6% (Table 2). Similarly, in the vineyard studies, conidial germination was inhibited from 4.7 to 19.5% while mycelial growth inhibition for the same isolates varied from 71.7 to 82.6% (Table 3). On the

Table 5. Frequency of dicarboximide-resistant isolates of *Botrytis cinerea* in table grapes in the Central Valley of Chile[y]

Year	No. of locations	*B. cinerea* isolates		
		No.	Resistant[z] (%)	
			Low level	High level
1988	51	101	2.0	0
1989	33	74	17.6	0
1990	72	182	13.7	0
1991	135	190	40.0	0
1992	46	122	64.0	0
1993	88	400	53.5	0.3
1994	63	630	74.9	0.6

[y] Samples were taken from table grape cv. Thompson Seedless or Flame Seedless near harvest (February–March).

[z] Mycelial growth inhibition of 50–94% for low-level resistance and <50% for high-level resistance on potato-dextrose agar amended with 10 mg/L of vinclozolin and incubated for 96 hr at 23 C (1,7,10). EC_{50} for high-level resistance = 11.0–18.2 mg/L of vinclozolin.

basis of these results, it appears that the ability to inhibit spore germination by DC fungicides is independent of the ability to inhibit mycelial growth of *B. cinerea*. This may be due to a possible coexistence of nuclei with sensitive (*Daf1*S), low resistance (*Daf1*LR), or high resistance (*Daf1*HR) alleles in different proportions in the heterokaryotic mycelium of *B. cinerea* isolates (7).

As previously reported (13,15,32), the ability to inhibit conidial germination may be rapidly lost after a few applications of DC fungicides or as soon as *Daf1*LR or *Daf1*HR genotypes become predominant in the population (7). However, our results demonstrate that the ability to arrest the mycelial growth of LLR isolates is only partially lost. This may explain the relatively high degree of control of gray mold that is still possible to achieve on table grapes after 10–15 yr of continuous use of these fungicides in most vineyards in the Central Valley of Chile. These results also suggest the inadequacy of relying on conidial germination test results alone for monitoring and measuring DC resistance in *B. cinerea* populations.

In agreement with previous reports, our results show that after a period of withholding DC fungicides, the population of *B. cinerea* reverts to greater sensitivity. This has been related to the lower fitness attributes of LLR isolates (1,3,6, 7,9,11,17,19,20,25,26). Some of these attributes, e.g., higher sensitivity to high osmolarity, were significantly lost in LLR isolates tested in this study.

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LITERATURE CITED

1. Beever, R. E., and Brien, H. M. R. 1983. A survey of resistance to the dicarboximide fungicides in *Botrytis cinerea*. N.Z. J. Agric. Res. 26:391-400.
2. Beever, R. E., and Byrde, R. J. W. 1982. Resistance to dicarboximide fungicides. Pages 101-107 in: Fungicide Resistance in Crop Protection. J. Dekker and S. G. Georgopoulos, eds. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands.
3. Beever, R. E., Laracy, E. P., and Pak, H. A. 1989. Strains of *Botrytis cinerea* resistant to dicarboximide and benzimidazole fungicides in New Zealand vineyards. Plant Pathol. 38:427-437.
4. Broome, J. C., Marois, J. J., and Latorre, B. A. 1992. Effect of leaf removal and fungicides on incidence and severity of Botrytis bunch rot in Thompson Seedless grapes in Chile. (Abstr.) Phytopathology 82:1075.
5. Carreño, I., and Alvarez, M. 1990. Determinación de razas resistentes de *Botrytis cinerea* de vides a fungicidas dicarboximidas. Agric. Tec. (Santiago) 50:298-303.
6. Davis, R. P., and Dennis, C. 1981. Properties of dicarboximide resistant strains of *Botrytis cinerea*. Pestic. Sci. 12:521-535.
7. Faretra, F., and Pollastro, S. 1993. Genetics of sexual compatibility and resistance to benzimidazole and dicarboximide fungicides in isolates of *Botryotinia fuckeliana (Botrytis cinerea)* from nine countries. Plant Pathol. 42:48-57.
8. Gouot, J.-M. 1988. Characteristics and population dynamics of *Botrytis cinerea* and other pathogens resistant to dicarboximides. Pages 53-55 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
9. Gullino, M. L., and Garibaldi, A. 1981. Biological properties of dicarboximide-resistant strains of *Botrytis cinerea* Pers. Phytopathol. Mediterr. 20:117-122.
10. Hoksbergen, K. A., and Beever, R. E. 1984. Control of low-level dicarboximide-resistant strains of *Botrytis cinerea* by dicarboximide fungicides. N.Z. J. Agric. Res. 27:107-111.
11. Hunter, T., Brent, K. J., Carter, G. A., and Hutcheon, J. A. 1987. Effects of fungicide spray regimes on incidence of resistant strains of *Botrytis cinerea*. Ann. Appl. Biol. 110:515-525.
12. Katan, T. 1982. Resistance to 3,5-dichlorophenyl-*N*-cyclicimide ('dicarboximide') fungicides in the grey mould pathogen *Botrytis cinerea* on protected crops. Plant Pathol. 31:131-141.
13. Katan, T., and Ovidia, S. 1985. Effect of chlorothalonil on resistance of *Botrytis cinerea* to dicarboximides in cucumber glasshouses. EPPO Bull. 15:365-369.
14. Latorre, B. A. 1986. Manejo de *Botrytis cinerea* en uva de mesa. Rev. Frutic. 7:75-83.
15. Latorre, B. A. 1992. Enfermedades de las Plantas Cultivadas. 3rd ed. Ediciones Universidad Católica, Santiago, Chile.
16. Leroux, P., and Clerjeau, M. 1985. Resistance of *Botrytis cinerea* Pers., and *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni to fungicides in French vineyards. Crop Prot. 4:137-160.
17. Löcher, F. J., Lorenz, G., and Beetz, K. J. 1987. Resistance management strategies for dicarboximide fungicides in grapes: Results of 6 years trials. Crop Prot. 6:139-147.
18. Lorenz, G. 1988. Dicarboximide fungicides: History of resistance development and monitoring methods. Pages 45-51 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
19. Lorenz, G., and Pommer, E.-H. 1985. Morphological and physiological characteristics of dicarboximide-sensitive and resistant isolates of *Botrytis cinerea*. EPPO Bull. 15:353-360.
20. Maraite, H., Meunier, S., Pourtois, A., and Meyer, J. A. 1980. Emergence in vitro and fitness of strains of *Botrytis cinerea* resistant to dicarboximide fungicides. Meded. Fac. Landbouwwet. Rijksuniv. Gent 45:159-167.
21. Northover, J., and Matteoni, J. A. 1986. Resistance of *Botrytis cinerea* to benomyl and iprodione in vineyards and greenhouses after exposure to the fungicides alone or mixed with captan. Plant Dis. 70:398-402.
22. Pak, H. A., Beever, R. E., and Laracy, E. P. 1990. Population dynamics of dicarboximide-resistant strains of *Botrytis cinerea* on grapevine in New Zealand. Plant Pathol. 39:501-509.
23. Pappas, A. C., and Fisher, D. J. 1979. A comparison of the mechanisms of action of vinclozolin, procymidone, iprodione, and prochloraz against *Botrytis cinerea*. Pestic. Sci. 10:239-246.
24. Pearson, R. C., and Goheen, A. C., eds. 1988. Compendium of Grape Diseases. American Phytopathological Society, St. Paul, MN.
25. Pommer, E.-H., and Lorenz, G. 1982. Resistance of *Botrytis cinerea* Pers. to dicarboximide fungicides—A literature review. Crop Prot. 1:221-230.
26. Pommer, E.-H., and Lorenz, G. 1987. Dicarboximide fungicides. Pages 91-106 in: Modern Selective Fungicides—Properties, Applications, and Mechanisms of Action. H. Lyr, ed. Longman Scientific & Technical, London.
27. Rewal, N., Colet-Smith, J. R., and Sealy-Lewis, H. M. 1991. Studies on resistance to dichlofluanid and other fungicides in *Botrytis cinerea*. Plant Pathol. 40:554-560.
28. Rustom, A., Latorre, B. A., and Lolas, M. 1989. Método para una correcta evaluación de la efectividad de nuevos fungicidas. Pages 149-164 in: Fungicidas y Nematicidas, Avances y Aplicabilidad. B. A. Latorre, ed. Facultad de Agronomía, Pontificia Universidad Católica de Chile, Santiago.
29. Sará, A. M., Latorre, B. A., Flores, V., and Roco, A. 1994. Situación de la resistencia de *Botrytis cinerea* en vid a fungicidas dicarboximídicos en Chile y pruebas de germinación de conidias como método de analisis. (Abstr.) Fitopatología 29:71-72.
30. SAS Institute. 1990. SAS/STAT User's Guide. Vol. 2. Version 6. 4th ed. SAS Institute, Cary, NC.
31. Shane, W. W., Joyner, T. G., and Powell, C. C. 1990. APPLICAL, RANDOMA, and MULTSTAT—Three microcomputer utilities for managing field trial experiments. Plant Dis. 74:333-334.
32. Vali, R. J., and Moorman, G. W. 1992. Influence of selected fungicide regimes on frequency of dicarboximide-resistant and dicarboximide-sensitive strains of *Botrytis cinerea*. Plant Dis. 76:919-924.
33. Wang, Z.-N., Coley-Smith, J. R., and Wareing, P. W. 1986. Dicarboximide resistance in *Botrytis cinerea* in protected lettuce. Plant Pathol. 35:427-433.