Frequency of Dicarboximide Resistant Strains of *Botrytis cinerea* in South African Table Grape Vineyards and Influence of Spray Schedules on Resistant Sub-Populations

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Between 1993 and 1995, 1139 isolates of *Botrytis cinerea* were collected from table grape vineyards in the Western and Northern Cape provinces and tested for sensitivity to dicarboximides (vinclozolin) and benzimidazoles (carbendazim). Overall the frequency of resistance to vinclozolin and carbendazim averaged 9,4 and 11,9%, respectively. Ninety-seven percent of the vinclozolin-resistant isolates were dual resistant to carbendazim. Fifty-nine percent of the isolates were from the Hexriver region; of these 7,7% were vinclozolin, and 12,6% carbendazim resistant. The Paarl region had the highest resistance incidence (37,7% vinclozolin and 41,5% carbendazim resistant). Only two of the 191 isolates tested from the Orange River region were vinclozolin resistant. ED_{50} values of ultra-low-level and low-level dicarboximide-resistant isolates ranged from 0,843 to 1,834 and 2,053 to 5,129 µg vinclozolin/m*l*, respectively. Vinclozolin-resistant strains were not abnormally osmotically sensitive. Monitoring of changes in the frequency of resistance in 10 commercial vineyards under high-, medium- and low-schedule dicarboximide programmes from 1993-1995 showed that the resistance frequency fluctuated from low (average 12,7%) during the winter to high (average 55,8%) during the growing season. Maximum levels of dicarboximide resistance were recorded during bunch closure. Distinct differences were, however, observed in the resistance frequency in individual vineyards. In four high-schedule dicarboximide vineyards resistance frequencies increased early in the season, prior to any dicarboximide applications. This finding suggests dual resistance between broad-spectrum fungicides and dicarboximides.

Botrytis bunch rot, caused by *Botrytis cinerea* Pers.: Fr., is the major cause of decay of stored grapes in South Africa (De Kock & Holz, 1991a; 1994). An integrated control programme, including canopy management and fungicide treatment, has been suggested for the past five years to improve the control of *B. cinerea* in vineyards, while lowering the traditional number of fungicide applications (De Kock & Holz, 1991a; 1994). Fungicide applications currently advocated include the fungicides, mancozeb and folpet, for the control of downy mildew during bloom and the green berry stages, followed by the more effective dicarboximides at véraison and before harvest. The rationale for these measures is that in the Western Cape province the disease is largely associated with infection during storage by inoculum present in bunches at véraison or at later stages (De Kock & Holz, 1991a; 1994).

Resistance in *B. cinerea* to dicarboximides is a well-recorded phenomenon worldwide (Pommer & Lorenz, 1982). Low-level resistant and highly resistant isolates of *B. cinerea* to dicarboximide fungicides have been described (Beever *et al.*, 1991; Latorre *et al.*, 1994). Dicarboximide fungicides have been used extensively during the past 10-15 years in most vineyards in the Paarl region, where conditions are often favourable after véraison for disease development. Although the risk of the pathogen developing resistance to dicarboximides is considered high, there have been no reports of major control failures in South African vineyards. This may be partially ascribed to the fact that in table grapes, loss of dicarboximide efficacy may not be easily recognizable, since sulphur dioxide fumigation is used to control *Botrytis* during storage (De Kock & Holz, 1991a; 1991b; 1994).

Growers are, however, concerned about the development and rapid dissemination of dicarboximide-resistant isolates among South African vineyards. The objective of this study was to determine the incidence of dicarboximide resistance in *B. cinerea* in vineyards of the Western and Northern Cape provinces, to characterize resistant isolates, and to examine changes in resistance frequency in vineyards under different dicarboximide schedules. Since benzimidazole fungicides have been used in South African table grape vineyards, and resistance to these compounds has been reported elsewhere (Delp, 1987), isolates were also tested for benzimidazole resistance.

MATERIALS AND METHODS

Fungicides and media: Vinclozolin (Ronilan 50 SC, BASF), iprodione (Rovral 25 SC, Rhône-Poulenc), procymidone (Sumisclex 25 SC, Agricura) and carbendazim (Bavistin 50 SC, BASF) were used in the study. Petri dishes of fungicide-amended potato-dextrose agar (PDA) (200 g sliced potatoes autoclaved in one liter distilled water, poured through cheesecloth, 20 g dextrose and 12 g agar added and autocalved) were prepared by adding fungicide suspensions from dilution series prepared in sterile distilled water to warm (50°C) molten PDA. The amended agar was mixed with a vortex mixer, poured into 9 cm plastic Petri dishes. Unless stated otherwise, rates are given as active ingredient.

Isolates: The export and marketing of table grapes from South Africa is mainly controlled by Unifruco (PO Box 505, Parc du Cap, Bellville 7535, South Africa). In order to regulate the quality of export grapes, the company enforced a Quality Evaluation Scheme, which requires that each producer's grapes be sampled for inspection prior to overseas shipment. Sample sizes are statiscially determined according to the number of boxes in each consignment. After sampling, the grapes are subjected to conditions simulating overseas shipment (4 weeks storage at -0,5°C followed by 1 week at 10°C) and fruit defects are determined at the end of the storage period. *Botrytis*-decayed bunches in Unifruco's Quality Evaluation Scheme from 1993 to 1995 were sampled and used for isolation. These samples from coded bunches were placed in individual plastic bags to prevent cross-contamination. The bags were sealed and kept at 22°C under diurnal light to promote sporulation. Single conidiophores of *B. cinerea* were selected under a dissecting microscope, placed on PDA (amended with 40 mg/*l* streptomycin sulfate), and incubated at 22°C for 72 h. Pure sub-cultures were obtained from hyphal tips growing on PDA to test sensitivity. Isolates selected for storage were stored on malt extract agar slopes at 5°C in the dark.

Dicarboximide resistance characterization: Resistance in *B. cinerea* to benzimidazole and dicarboximide fungicides was determined according to the protocols of the Fungicide Resistance Action Committee (FRAC) (Löcher & Lorenz, 1991). The level of resistance in *B. cinerea* to different dicarboximide fungicides and occurrence of cross-resistance were determined on 262 isolates obtained during the 1993 harvest season. Mycelial growth sensitivity of each isolate to dicarboximide fungicides was firstly determined on PDA amended with 0 (control); 0,1; 0,5; 1,3 or 5 µg vinclozolin (a.i)/m*l*. Mycelial plugs (5 mm in diameter) were taken from the active-growing colony margins of the pure cultures and placed in the centres of each of 15 plates containing the range of vinclozolin concentrations (three plates per concentration) and on three non-amended PDA plates. The plates were incubated for 36 h at 22°C and the radial mycelial growth determined. Each colony's diameter was measured twice perpendicularly for each of the three replicates and the control. The fungicide concentration that inhibited colony growth of the isolates by 50% (EC_{50} value) compared to the control was determined by a regression analysis of the log-inhibition. Ward's minimum variance cluster analysis was used to identify dicarboximide resistant subgroups (Ward, 1963).

Isolates resistant to vinclozolin were tested for cross-resistance to iprodione and procymidone. Tests were performed in triplicate on PDA amended with either iprodione, or procymidone, at concentrations given for vinclozolin. The cultures were incubated at 22°C for 36 h before determining radial growth of mycelium. Linear regression was done on the data, using SAS systems.

Fungicide resistance survey: The mycelial growth sensitivity to benzimidazole and dicarboximide fungicides of all the *B. cinerea* isolates sampled from 1993 to 1995 were determined on PDA amended with discriminatory levels (as determined in the dicarboximide resistance characterization) of 3 µg vinclozolin/m*l* or 5 µg carbendazim/m*l*, respectively. Mycelial plugs (5 mm in diameter), taken from the active-growing colony margins of the pure cultures, were placed on each of three plates containing either vinclozolin, or carbendazim, and on one non-amended PDA plate. The plates were incubated for 36 h at 22°C and the radial mycelial growth determined. Since a discriminatory concentration of fungicide was used, isolates were designated resistant if they grew on the control and fungicide-amended plates.

Osmotic sensitivity of dicarboximide-resistant isolates: Earlier work attributed reduced fitness in dicarboximide resistant isolates to an abnormal osmotic sensitivity (Beever, 1983; Beever & Brien, 1983; Leroux & Clerjeau, 1985). The degree of osmotic sensitivity of isolates designated as sensitive or resistant by the 3 µg vinclozolin/m*l* discriminatory test was determined on PDA amended with 0,68 M NaCl (Beever, 1983). Mycelial plugs (5 mm in diameter) were taken from the active-growing colony margins of pure cultures of 50 sensitive and 50 resistant isolates and placed in the centres of NaCl-amended and non-amended PDA plates. The plates were incubated for 36 h at 22°C and the radial mycelial growth determined. Tests were performed in triplicate. The experiment was repeated on Difco PDA with another 10 sensitive, 12 ultra-low-level resistant and 10 low-level resistant isolates, previously characterized (as part of this study) for relative sensitivity to five concentrations of vinclozolin.

Frequency of dicarboximide resistant isolates in vineyards: Trials were established in 1993 in 10 commercial vineyards at five different localities in the Paarl region. The vineyards were selected on the basis of the resistance survey done during the 1993 harvest season and the number of dicarboximides applied per season (high-, medium- and low-dicarboximide schedule). Two cultivars, Waltham Cross and Dan-ben-Hannah, were used at each locality. Localities were Simondium, Central Paarl A, Central Paarl B, Northern Paarl A and Northern Paarl B. Vineyard blocks ranged from 1 to 5 ha and the vines were trained to a slanting trellis at 3 x 1,5 m spacing. All vines were micro-spray irrigated, except those at Northern Paarl B, which were drip irrigated. Canopy management and bunch preparation were done according to the guidelines of Van der Merwe *et al.* (1991). A recommended programme for the control of downy and powdery mildew (De Klerk, 1985) was followed in all vineyards. Sprays against downy mildew started at 10-15 cm shoot length and were applied every 14 days until pea-berry stage. Fungicides used were folpet (Folpet 50 WP, Zeneca), fosetyl-Al/mancozeb (Mikal M 44/26 WP, Rhône-Poulenc), mancozeb (Dithane M45 80 WP, Zeneca) and mancozeb/metalaxyl (Ridomil MZ 60/10 WP, Novartis). Powdery mildew fungicides such as penconazole (Topaz 10 EC, Novartis), pyrifenox (Dorado 48 EC, Novartis) and triadimenol (Bayfidan 25 EC, Bayer) started at 2-5 cm shoot length and were applied every 14 days until 3 weeks before harvest. In all vineyards folpet was applied as 2-4 sprays from 2-5 cm shoot length until bloom for the control of *Phomopsis viticola.*

B. cinerea samples were obtained from mummified bunches and/or leaves from vines in June (pre-prune stage), from pre-

bloom bunches and young vegetative growth from active-growing canes in October (pre-bloom stage), from bunches with abscised flowers, flower remnants, and young vegetative growth in November (pea-berry stage) and December (bunch closure stage) and from symptomatic berries at harvest in February. Ten samples were obtained from each vineyard at each sampling stage.

Sporulation on plant material was induced in humidified plastic bags and the mycelial growth sensitivity to dicarboximide fungicides determined using the discriminatory dose method described previously. The percentage resistant isolates as a proportion of the total number of isolates tested was calculated for each vineyard.

RESULTS

Dicarboximide resistance characterization: Mycelial growth of 212 of the 262 *B. cinerea* isolates used was nearly completely inhibited (90,6%, SD 5.1) at 0,5 µg vinclozolin/m*l* (Table 1). Fifty isolates grew at 1 µg vinclozolin/m*l* and were completely cross-resistant to iprodione and to procymidone. Growth of these isolates was negatively inhibited at 0,1 µg/m*l*.

TABLE 1

Growth of *Botrytis cinerea* isolates, obtained from symptomatic table grape bunches, on PDA amended with dicarboximide fungicides.

Fungicide concentration (µg a.i./m*l*)	Inhibition (%) relative to control			
	Sensitive isolates[a]	Resistant isolates		
	Vinclozolin	Vinclozolin	Iprodione	Procymidone
0	0	0	0	0
0,1	57,2 (12,5)[b]	-4,3 (14,7)	-1,2 (4,6)	-0,8 (14,3)
0,5	90,6 (5,1)	4,3 (16)	6,8 (7,9)	0,9 (14,7)
1	100	29,1 (11,5)	27,3 (13,6)	6,2 (17,2)
3	100	74,6 (5)	64,4 (4,9)	49,4 (17,7)
5	100	86,8 (5,1)	78,9 (4,9)	59,9 (12,6)

[a] Two hundred and sixty-two isolates tested; 212 dicarboximide sensitive, 50 dicarboximide resistant.

[b] Numbers in parenthesis are the standard deviation of the mean.

Ward's minimum variance cluster analysis (Ward, 1963) of dicarboximide sensitivity data divided the 50 resistant isolates into an ultra-low-level and a low-level resistant group for each fungicide (Table 2). ED_{50}-values of ultra-low-level and low-level vinclozolin-resistant isolates ranged from 0,843 to 1,834 and 2,053 to 5,129 µg/m*l*, respectively. The corresponding values for iprodione were 0,782 to 1,727 and 2,194 to 3,074 µg/m*l* respectively, and for procymidone 0,721 to 2,589 and 3,502 to 10,695 µg/m*l*, respectively.

TABLE 2

Range of ED_{50}-values of *Botrytis cinerea* isolates in three dicarboximide resistance classes on PDA amended with different dicarboximide fungicides.

Resistance class	Number of isolates	Mean ED_{50} value		
		Vinclozolin	Iprodione	Procymidone
Sensitive	212	0,001-0,291	-- [a]	---
Ultra-low-level	34	0,843-1,834	0,782-1,727	0,721-2,589
Low-level	16	2,053-5,129	2,194-3,074	3,502-10,695

[a] Not tested.

Resistance survey: A total of 1139 *B. cinerea* isolates were obtained during the period 1993-1995 from symptomic bunches collected from the Unifruco Quality Evaluation Scheme. Overall, the frequency of resistance to vinclozolin and carbendazim averaged 9,4 and 11,9%, respectively (Table 3). Ninety-seven percent of the vinclozolin-resistant isolates were also resistant to carbendazim. Fifty-nine percent of the isolates were from the Hexriver region; of these 7,7% were vinclozolin, and 12,6% carbendazim resistant. The Paarl region had the highest resistance incidence (37,7% vinclozolin and 41,5% carbendazim resistant). Of the 191 isolates tested from the Orange River region only two were vinclozolin resistant.

TABLE 3

Mean frequency of resistance to vinclozolin and carbendazim in *Botrytis cinerea* isolates obtained from symptomatic table grape bunches collected during 1993-1995 from Unifruco's Quality Control Evaluation Scheme.

Region	Number of isolates	Resistance frequency (%)		
		Vinclozolin	Carbendazim	Vinclozolin & Carbendazim
Hexriver valley	672	7,7	12,6	6,4
Paarl	77	37,7	41,5	37,7
Wellington	24	12,5	11,1	11,1
Robertson	29	10,3	8,7	8,7
Piketberg	25	0	0	0
Orange River	191	1,0	0,5	0,5
Porterville	71	9,9	12,7	9,9
Riebeeck-Kasteel	24	12,5	0	0
Other	26	23,1	27,0	18,9
Mean		9,4	11,9	7,7

Osmotic sensitivity: The mycelial growth rate of the isolates designated as sensitive and resistant by the 3 µg vinclozolin/ml discriminatory test on NaCl-amended PDA did not differ significantly (Table 4). Radial growth of the vinclozolin-resistant isolates on non-amended PDA was, however, significantly slower than that of the vinclozolin-sensitive isolates. No difference in colony morphology of the two types of isolates was observed on any of the media. Similar data were recorded with the 10 sensitive, 12 ultra-low-level resistant and 10 low-level resistant isolates (Table 5).

TABLE 4

Growth of 50 dicarboximide-sensitive and 50 dicarboximide-resistant isolates of *Botrytis cinerea* on PDA amended with 0.68 M sodium chloride.

Type isolate	Colony diameter (mm)[x]		Inhibition(%)
	PDA	PDA+NaCl	
Sensitive	40,5	20,1	50,6
Resistant	32,1	16,4	49,2

[x] Colony diameters measured perpendicularly after 36 h growth at 22°C.

TABLE 5

Mean colony diameters of *Botrytis cinerea* isolates in three dicarboximide sensitivity classes on Difco PDA amended with 0.68 M sodium chloride.

Sensitivity class	Number of isolates	Colony diameter (mm)[x]		
		Difco PDA	Difco PDA + NaCl	Inhibition (%)
Sensitive	10	28,1 (2,7)	16,9 (2,4)	39,9
ULR[y]	12	26,6 (2,3)	16,5 (2,3)	37,9
LR[z]	10	27,2 (2,9)	17,0 (2,8)	37,6

[x] Colony diameters measured perpendicularly after 24 h growth at 22°C. Mean values are given with standard deviation of means given in parenthesis.

[y] ULR = Ultra-low-level resistant.

[z] LR = Low-level resistant.

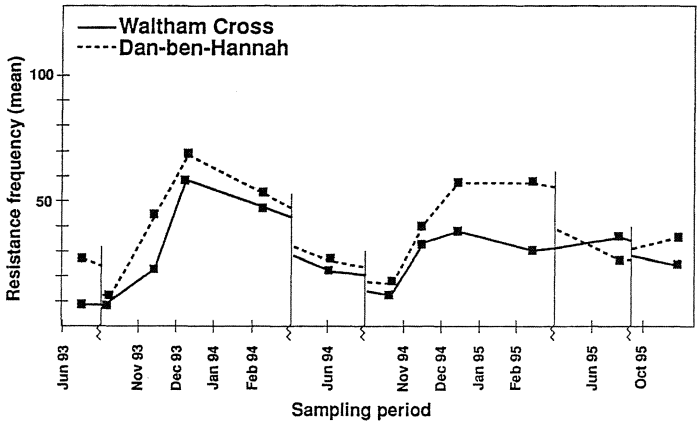

FIGURE 1

Mean dicarboximide resistance frequency of *Botrytis cinerea* isolates collected at different growth stages from five Dan-ben-Hannah and five Waltham Cross vineyards in the Western Cape province during the period from 1993-1995. Oct. = pre-bloom; Dec. = bunch closure; Feb. = harvest.

Frequency of dicarboximide-resistant isolates in selected vineyards: The data from all trials are summarized in Fig. 1, where the mean resistance frequency in the Dan-ben-Hannah and Waltham Cross vineyards is plotted for each sampling period. Overall, the resistance frequency decreased during winter and increased during the growing season; it reached a minimum value at the pre-bloom stage (average 20,8 and 14,8% for Dan-ben-Hannah and Waltham Cross, respectively) and a maximum level (average 61,7 and 48% for Dan-ben-Hannah and Waltham Cross, respectively) at bunch closure. Distinct differences were, however, observed in the resistance frequency of individual vineyards. Under the high-dicarboximide schedule (Fig. 2), the resistance frequency tended to remain at a relatively high level for each sampling, except for 1993 when zero levels were recorded at pre-prune in Simondium (Waltham Cross) and Central Paarl B (Dan-ben-Hannah and Waltham Cross), and at pre-bloom in Northern Paarl A (Dan-ben-Hannah and Waltham Cross) and Central Paarl A (Dan-ben-Hannah). During the bunch closure and harvest stages of each season, resistance frequencies higher than 50% were recorded in all these vineyards. In vineyards at Simondium and Northern Paarl A, an increase in resistance frequency at the pre-bloom stage was recorded before dicarboximides were applied. In vineyards with a medium-schedule programme (Fig. 3), dicarboximide resistant strains were absent during the 1995 pre-bloom period. However, resistance frequencies tended to increase during each season after the first dicarboximide application at bloom. Maximum levels were recorded during bunch closure. A similar trend was

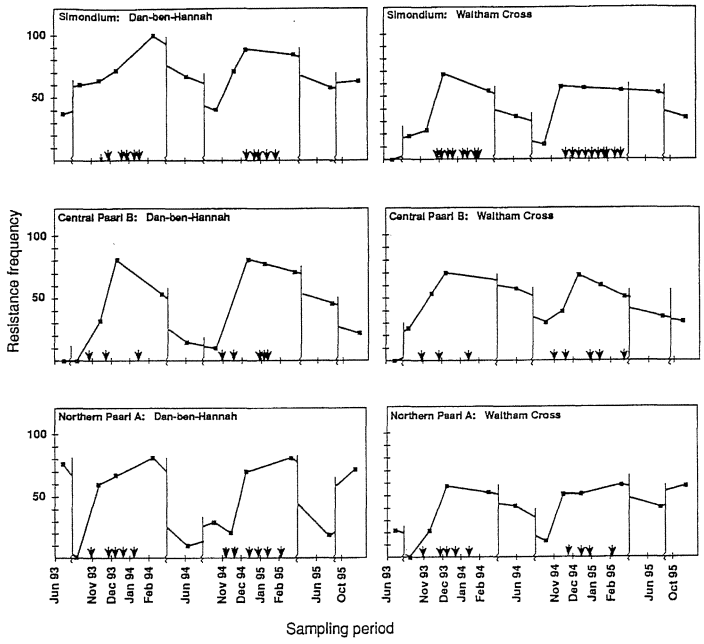

FIGURE 2

Dicarboximide resistance frequency of *Botrytis cinerea* isolates in table grape vineyards under a high dicarboximide schedule during the period 1993-1995. Oct = pre-bloom; Dec = bunch closure; Feb = harvest. Arrowheads indicate the dicarboximide applications.

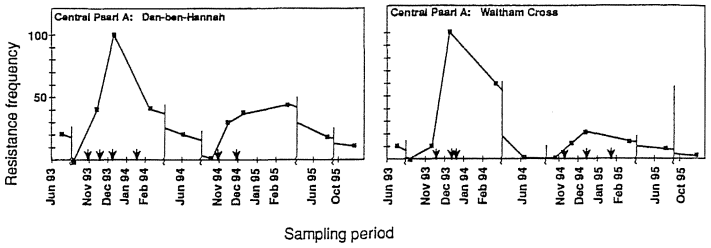

FIGURE 3

Dicarboximide resistance frequency of *Botrytis cinerea* isolates in table grape vineyards under a medium dicarboximide schedule during the period 1993-1995. Oct. = pre-bloom; Dec. = bunch closure; Feb. = harvest. Arrowheads indicate the dicarboximide applications.

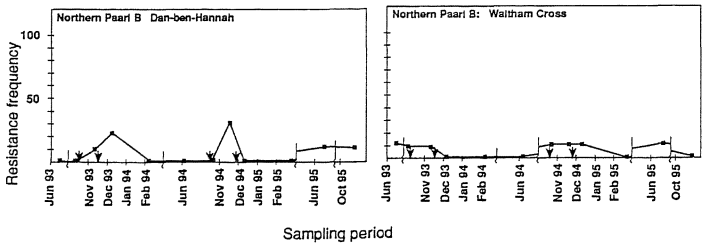

FIGURE 4

Dicarboximide resistance frequency of *Botrytis cinerea* isolates in table grape vineyards under a low dicarboximide schedule during the period 1993-1995. Oct. = pre-bloom; Dec. = bunch closure; Feb. = harvest. Arrowheads indicate the dicarboximide applications.

found in vineyards under a low-schedule programme (Fig. 4). In these vineyards, however, the resistance frequency remained at a very low level.

The effect of the number of cumulative dicarboximide applications under each dicarboximide programme on the mean resistance frequency recorded for each vineyard over the three-year period of study is given in Table 6. The mean resistance frequency in the two Northern Paarl B vineyards was kept below 7% under the low-dicarboximide schedule comprising of six cumulative sprays.

TABLE 6

Mean resistance frequency of *Botrytis cinerea* isolates in table grape vineyards in the Western Cape province under different dicarboximide (DC) schedules over the period June 1993 - October 1995.

Locality	Cultivar	Number of dicarboximide applications	Mean resistance frequency
High DC schedule			
Simondium	Waltham Cross	18	31,9 (22,0)[a]
	Dan-ben-Hannah	15	66,7 (18,2)
Northern Paarl A	Waltham Cross	9	37,0 (19,8)
	Dan-ben-Hannah	11	41,7 (31,5)
Central Paarl B	Waltham Cross	8	41,8 (21,3)
	Dan-ben-Hannah	7	34,4 (33,1)
Medium DC schedule			
Central Paarl A	Waltham Cross	6	17,6 (29,8)
	Dan-ben-Hannah	5	21,6 (16,7)
Low DC schedule			
Northern Paarl B	Waltham Cross	4	6,6 (5,2)
	Dan-ben-Hannah	4	6,9 (10,2)

[a] Numbers in parenthesis are standard deviation of the mean.

DISCUSSION

Isolates of *B. cinerea* resistant to dicarboximide and benzimidazole fungicides have not been reported from South African vineyards previously. This survey, which included isolates from most table grape vineyards in South Africa, showed that resistance to dicarboximide and benzimidazole fungicides is present in *B. cinerea* populations from most table grape-growing regions. As a general conclusion, the frequencies of dicarboximide and benzimidazole resistance within vineyards in the Western and Northern Cape provinces are lower than those reported from various grape-growing regions in Europe (Leroux & Clerjeau, 1985; Löcher *et al.*, 1987), New Zealand (Beever *et al.*, 1989), and Canada (Northover, 1988). Furthermore, benzimidazole-resistant strains were still found in vineyards in nearly all the regions despite the limited use of benzimidazole fungicides in table grape vineyards after the introduction of dicarboximides in 1978 (Aggenbach & Marais, 1978). This conforms with the distribution pattern of such strains in other countries (Leroux & Clerjeau, 1985; Northover & Matteoni, 1986; Beever *et al.*, 1989) and indicates that local benzimidazole-resistant strains of *B. cinerea* are just as fit as benzimidazole-sensitive strains since they retained their proportion in the population after benzimidazole usage had ceased (Schüepp & Küng, 1981).

The highest incidence of resistance to both fungicide groups was recorded in the Paarl region. The climate of the Paarl region usually favours the development of *B. cinerea* during bloom and harvest (mean temperature: min 10,1°C; max 21,8°C; humidity 43,8-84,4% RH; rainfall 650 mm). Dicarboximides are used more extensively in the Paarl region than in other regions. High selection pressures are therefore applied on the *B. cinerea* populations in table grape vineyards in the Paarl region, which would enhance the development of dicarboximide-resistant strains (Dekker, 1993). Resistance to both fungicide groups in the Orange River region was very low, despite the fact that a large number of samples were screened from this region and that mainly early-season Sultana Seedless, which is very susceptible to *B. cinerea*, are grown. Low rainfall (mean 110 mm), low humidity (mean 23,7-69,8% RH) and high mean day temperatures (mean min 14,6°C, mean max 31,9°C), which do not favour the development of *B. cinerea* (Jarvis, 1980), usually prevail during most of the

growing season. Less dicarboximides are used, and it can be assumed that few generation cycles of the pathogen would occur in Orange River vineyards, thereby reducing the selection pressure in favour of resistance build-up in the *B. cinerea* population.

The level of resistance exhibited by the local strains is comparable to that of moderate resistant strains found internationally (Katan, 1982; Beever & Brien, 1983; Northover, 1983; Leroux & Clerjeau, 1985; Beever *et al.,* 1989; Latorre *et al.,* 1994). The ED_{50}-values for vinclozolin were grouped between 0,843 and 5,129 µg/m*l*. The cluster analysis divided the isolates into two groups, ultra-low-(0,843 to 1,834 µg/m*l*) and low-level resistant (2,053 to 5,129 µg/m*l*), which agrees with the division found in New Zealand (Beever *et al.,* 1989).

The local resistant strains were not abnormally osmotically sensitive as reported for resistant strains elsewhere (Beever, 1983; Beever & Brien, 1983; Leroux & Clerjeau, 1985). The reason for this might be that the local strains are moderately resistant and not high-level resistant or laboratory induced. Beever & Brien (1983) reported abnormal sensitivity to high osmotic pressure in low-level resistant strains. Although the local resistant strains were not abnormally osmotically sensitive, we observed a marked reduction in fitness, which was observed as reduced mycelial growth rate on PDA of resistant strains compared to sensitive strains. Similar reduced growth rates in dicarboximide-resistant strains were reported in Israel (Katan, 1982), Canada (Northover, 1983) and Spain (Fraile *et al.,* 1986).

The drastic decline in dicarboximide resistance in *B. cinerea* in the Western Cape vineyards during winter can be attributed to the climate, which is moderate (mean temperature: min 10,1°C; max 21,8°C) and wet (humidity 43,8-84,4% RH; rainfall 650 mm). Several workers reported reduced fitness in dicarboximide-resistant strains from England (Hunter *et al.,* 1987), Germany (Löcher *et al.,* 1987), Chili (Latorre *et al.,* 1994), Israel (Katan & Ovadia, 1985), Canada (Northover, 1988) and New Zealand (Pak *et al.,* 1990). Manning & Brook (1991) found that resistant strains grew slower than sensitive strains at low temperatures. During extremely cold winters, in some parts of Europe, for example, the *Botrytis* populations are subjected to extremely low temperatures that inhibit mycelial growth. In vineyards in the Western Province and Northern Cape, mycelial growth and sporulation are not completely inhibited during winter and competition between sensitive and resistant strains should occur. The superior fitness of sensitive strains would enable that population to proliferate faster than the resistant population in the absence of dicarboximides during the winter months. As a result the decline in resistance frequency during winter in the Western Province and Northern Cape should be more pronounced than in European conditions (Leroux & Clerjeau, 1985) and in New Zealand vineyards (Beever *et al.,* 1989).

According to Beever *et al.* (1991), the resistance incidence fluctuates around a balance value that is determined by the number of dicarboximide applications and the climatic conditions. In this study an early increase in the resistance frequency that reaches maximum levels at bunch closure occurred in vineyard showing a high resistance balance value. This phenomenon can be ascribed to the dynamics of the *B. cinerea* population under the local climate, which usually favours the pathogen from the pre-bloom to the pea-size stage. Research in New Zealand (Beever *et al.,* 1991) and Germany (Löcher *at el.,* 1987) indicates that selection for dicarboximide-resistant strains of *B. cinerea* in wine grapes occurs most rapidly during periods that are most favourable for development of the pathogen. Pollen and flower debris provide an excellent nutrient source for the development of *B. cinerea* at flowering, and colonization of aborted flowers and floral debris within bunches has been recorded (Gessler & Jermini, 1985; Nair & Parker, 1985; Northover, 1987; De Kock & Holz, 1994). This nutrient source may enhance the proliferation of dicarboximide-resistant strains in aborted flowers and flower debris in dicarboximide-sprayed bunches during bloom and early fruit set. Iprodione and procymidone are ineffective in eradicating *B. cinerea* from aborted flowers and dead flower parts during these growth stages (De Kock, 1989; De Kock & Holz, 1994). Aborted flowers and flower remnants in clusters removed for sampling during the pea-size stage might have been colonized by dicarboximide-resistant strains, whereas these parts might have been absent in bunches sampled later. This is substantiated by the decline in resistance frequency recorded for each season after bunch-closure in most vineyards. De Kock & Holz (1994) found that dead flowers colonized by *B. cinerea* occurred in bunches of the table grape Barlinka until late pea size, but not at véraison. This finding was ascribed to table grape bunches being looser, thereby allowing abscised floral parts to drop from the bunch.

In vineyards under high dicarboximide schedules in Simondium and Northern Paarl A, an increase in dicarboximide-resistance frequency was recorded before a dicarboximide was applied at bloom. In all these vineyards 3-4 early-season folpet applications were made to control *P. viticola.* Leroux & Fritz (1983) reported that dicarboximide-resistant strains remain sensitive to sulphenimides, such as folpet and dichlofluanid, and the dithiocarbamates. Barak & Edgington (1984) found significant cross-resistance between folpet, captab, captafol, etem, thiram, and chlorothalonil, but observed no cross-resistance between these compounds and iprodione. Cross-resistance between dichlofluanid-resistant isolates and dicarboximides was reported by Washington *et al.* (1992) and strains resistant to both dichlofluanid and dicarboximides were reported by Raposo *et al.* (1996). Cross-resistance between other members of the sulphenimide group has been reported (Barak & Edgington, 1984). The increase in dicarboximide resistance frequency after successive folpet applications in vineyards with high dicarboximide resistance balance values therefore strongly suggests dual resistance between this broad-spectrum fungicide and the dicarboximides in these vineyards.

The programme currently recommended to growers in the Western Cape is a low-dicarboximide schedule, where two dicarboximide applications are restricted to the period from véraison (De Kock & Holz, 1991a, 1994). The rationale for these measures is that in the Western Cape, contrary to some other countries (McClellan & Hewitt, 1973; Sparapano *et al.,* 1981; Nair & Parker, 1985), no clear relation exits between the occurrence of the pathogen in aborted flowers and floral remnants during bloom

and post-harvest *Botrytis* bunch rot. After the flowering period, unripe, undamaged berries are rarely colonized by *B. cinerea*, and the disease is largely associated with infection during storage by inoculum present in bunches at véraison or at later stages (De Kock & Holz, 1991a; 1994). Our data on the early-season increase in resistance in vineyards exhibiting high, medium and low dicarboximide resistance balance values, and observations on disease prevalence, support this recommendation. However, the possibility that dicarboximide-resistant isolates might develop dual resistance to broad-spectrum fungicides suggests that the latter fungicides should also be used judiciously.

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