Incomplete Cross-Resistance to Folpet and Iprodione in *Botrytis cinerea* from Grapevine in South Africa

P.H. Fourie* and G. Holz

Department of Plant Pathology, University of Stellenbosch, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa

Submitted for publication: February 2000
Accepted for publication: April 2001
Key words: *Botrytis cinerea*, folpet, dicarboximides, table grapes

The sensitivity to folpet of *Botrytis cinerea* isolates obtained from table grape vineyards in the Western Cape province of South Africa with a known history of dicarboximide (DC) resistance and high-schedule DC and folpet programmes was investigated. In the Simondium vineyards, 61% of the *B. cinerea* isolates from Dan-ben-Hannah and 20% of the isolates from Waltham Cross were resistant to iprodione. In the Northern Paarl vineyards, 95% of the isolates from Dan-ben-Hannah and 95% of the isolates from Waltham Cross were designated resistant. In the case of the iprodione-sensitive isolates from vineyards in Simondium, folpet EC_{50} values ranged from 4.9 to 29.1 µg/mL for the Dan-ben-Hannah and 15.0 to 43.5 µg/mL for the Waltham Cross sub-populations, respectively. Folpet EC_{50} values of the iprodione-resistant isolates, on the other hand, ranged from 19.7 to above 100 µg/mL for the Dan-ben-Hannah subpopulation. In the Northern Paarl subpopulations, where the isolates were predominantly iprodione-resistant, folpet EC_{50} values of the latter isolates ranged from 21.5 to above 100 µg/mL. Similar shifts in folpet sensitivity were displayed by ultra-low- and low-level DC-resistant *B. cinerea* isolates obtained from other regional subpopulations. The results indicated incomplete cross-resistance between iprodione and folpet. This finding suggests that early increases in DC resistance frequencies in *B. cinerea*, observed prior to DC application in vineyards under the high-schedule DC and folpet programmes, can be attributed to incomplete cross-resistance to these fungicides in sub-populations of the pathogen.

Resistance in *Botrytis cinerea* to dicarboximide fungicides (DC) is a well-recorded phenomenon world-wide (Pommer & Lorenz, 1982, 1995; Fourie, 1996). In the Western Cape province of South Africa, maximum levels of DC resistance in table grape vineyards occurred during bunch closure (Fourie & Holz, 1998). Resistance incidences in vineyards under high-, medium- and low-schedule DC programmes furthermore fluctuated from low (average 12.7%) over the winter period to high (average 55.8%) during the growing season. The winterly decline in resistance incidence was attributed to the reduced ecological competence of the resistant sub-populations, combined with moderate winter temperatures, which would allow competition between resistant and sensitive sub-populations (Fourie, 1996; Fourie & Holz, 1998). In some of the high DC-schedule vineyards resistance frequencies increased early in the season prior to the application of DCs (Fourie & Holz, 1998). Given the reduced fitness of DC-resistant strains, it was suggested that this phenomenon might be attributed to the selection pressure exerted on the *B. cinerea* population by other fungicides applied during the pre-flowering stage. Folpet is primarily applied at the pre-blossom stage in local vineyards against *Phomopsis viticola*, and also to support the control of *B. cinerea* and *Plasmopara viticola* (Nel *et al.*, 1999; Vermeulen, 1999). Folpet, along with related fungicides like captab and dichlofluanid, is a broad-spectrum fungicide in the sulphenimide group (Leroux & Fritz, 1984). Barak and Edgington (1984b) observed cross-resistance in *B. cinerea* amongst captab, folpet, captafol, etem, thiram and chlorothalonil, but not between these compounds and iprodione. Cross-resistance was, however, found between DCs and dichlofluanid (Hunter *et al.*, 1987; Washington *et al.*, 1992; Raposo *et al.*, 1996). DC-resistant strains of the pathogen remained sensitive to dithiocarbamates (e.g. thiram), sulphenimides (e.g. captan, dichlofuanid and folpet) and chlorothalonil (Leroux & Fritz, 1984). In French vineyards, an increase in DC resistance frequencies of *B. cinerea* was reported after the application of folpet or dichlofluanid (Leroux & Clerjeau, 1985). A similar trend was observed (Hunter *et al.*, 1987) with dichlofluanid on strawberries. The increase in DC resistance in *B. cinerea* populations displaying high DC resistance balance values after successive folpet applications (Fourie & Holz, 1998) therefore strongly suggests dual resistance in the pathogen to this broad-spectrum fungicide and the DCs.

The aim of this study was to determine the sensitivity to folpet of *B. cinerea* isolates obtained from vineyards with a known history of DC resistance and high-schedule DC and folpet programmes.

MATERIALS AND METHODS

Vineyards

Four vineyards previously subjected to high-schedule DC and folpet programmes (Table 1) were selected in autumn 1998 in two different localities in the Paarl region, Simondium and Northern Paarl. The *B. cinerea* population in these vineyards displayed high DC resistance balance values and increased DC resistance frequencies prior to the application of these fungicides during 1993-1995 (Fourie & Holz, 1998). Two cultivars, Waltham Cross and Dan-ben-Hannah, were used at each locality. Vineyard blocks ranged from 1 to 5 ha and the vines were trained to a slanting trellis at 3 x 1.5 m spacings. All vines were micro-irrigated. Canopy

*Present address: ARC Infruitec-Nietvoorbij, Private Bag X5026, 7599 Stellenbosch, South Africa.

TABLE 1

Number of dicarboximide and folpet applications applied during five consecutive seasons in Dan-ben-Hannah and Waltham Cross table-grape vineyards in two different localities.

Location	Cultivar	Number of applications per season									
		1993/94		1994/95		1995/96		1996/97		1997/98	
		F[x]	DC[y]	F	DC	F	DC	F	DC	F	DC
Simondium	Dan-ben-Hannah	4	6	4	5	4	4	0	4	0	4
	Waltham Cross	3	8	3	9	3	4	0	4	0	4
Northern Paarl	Dan-ben-Hannah	3	5	3	5	4	5	5	6	4	3
	Waltham Cross	3	5	3	5	4	5	5	6	4	3

[x]F = Folpet

[y]DC = Dicarboximide

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 size. 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). Applications against powdery mildew started at 2-5 cm shoot length and were applied every 14 days until 3 weeks before harvest. Fungicides used were penconazole (Topaz 10 EC, Novartis), pyrifenox (Dorado 48 EC, Novartis) and triadimenol (Bayfidan 25 EC, Bayer). In all vineyards an additional programme was followed for the control of *Phomopsis viticola*. Folpet was applied as 2-4 sprays from 2-5 cm shoot length until bloom.

Isolates

Botrytis cinerea was obtained from symptomatic berries or leaves collected in the selected vineyards during autumn. The plant material was placed in individual polyethylene bags to prevent cross-contamination. The bags were sealed and kept at 22°C under diurnal light to stimulate sporulation. Single conidiophores of *B. cinerea* were selected under a dissecting microscope, placed on potato dextrose agar (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 streptomycin amended PDA. Isolates selected for further use were kept on malt extract agar slopes at 5°C in the dark.

Isolates that were obtained from other regional sub-populations and characterised for DC sensitivity (Fourie, 1996; Fourie & Holz, 1998) were included for comparison. These isolates were selected from a culture collection consisting of *B. cinerea* isolates obtained from various South African table grape vineyards. Fourteen sensitive (EC_{50} values 0.001 – 0.3 µg a.i./mL), 10 ultra-low- (EC_{50} values 0.8 – 1.8 µg a.i./mL) and five low-level (EC_{50} values 2.1 – 5.1 µg a.i./mL) DC-resistant *B. cinerea* isolates were used.

Fungicide sensitivity tests

Resistance to iprodione (Rovral 25 SC, Rhône-Poulenc) in *B. cinerea* from the selected vineyards was determined according to the protocols of the Fungicide Resistance Action Committee (FRAC) (Löcher & Lorenz, 1991). The mycelium growth sensitivity of the isolates was determined on PDA amended with 3 µg iprodione/mL. Mycelium plugs (5 mm in diameter) were taken from the actively growing colony margins of the pure cultures and placed on each of three non-amended plates, as well as on three iprodione-amended plates. The plates were incubated for 36 h at 22°C and the radial mycelium growth determined. Since a discriminatory concentration of fungicide was used, an isolate was designated resistant if it grew on the control and fungicide-amended plates and sensitive if it grew only on the control plates (Fourie & Holz, 1998).

Two methods were used to determine the degree of sensitivity of the different isolates to folpet (Folpan 50 SC, Maghteshim-Agan), i.e. mycelium growth and spore germination. Mycelium growth was determined on PDA amended with 0 (control), 2.5, 5, 10, 25 and 50 µg folpet (a.i.)/mL. Mycelium plugs (5 mm in diameter) were taken from the actively growing colony margins of the pure cultures and placed in the centre of plates containing the range of folpet concentrations (three plates per concentration) and on three non-amended plates. The plates were incubated for 36 h at 22°C and the radial mycelium growth determined. Colony diameter was measured twice perpendicularly and percentage inhibition calculated. The fungicide concentration that inhibited colony growth of the isolates by 50% compared to the control (EC_{50} value) was determined by regression analysis. Ward's minimum variance cluster analysis was used to identify folpet resistant subgroups (Ward, 1963). Fifteen isolates with folpet EC_{50} values for mycelium growth ranging from 4.93 to above 100 µg folpet/mL were selected for the spore germination tests. Conidia were washed with sterile deionised water from sporulating, 2-week-old cultures of the selected isolates. Small aliquots (0.5 mL) of conidial suspension were spread-inoculated onto PDA plates amended with 0, 0.025, 0.05, 0.1, 0.25, 0.5, 1 or 2.5 µg folpet (a.i.)/mL. The inoculated plates were incubated for 18 h at 22°C. The number of germinated (germ tube longer than conidium diameter) and non-germinated conidia (germ tube shorter than conidium diameter) were counted by using a microscope (200x magnification), and expressed as percentage germination. EC_{50} values were determined and correlated with the same isolates' EC_{50} values for mycelium growth, using Pearson's correlation (Snedecor & Cochran, 1967).

RESULTS

The percentage *B. cinerea* isolates in each vineyard designated resistant to iprodione are given in Table 2. In the Simondium vineyards, 61% of the isolates from Dan-ben-Hannah and 20% of the isolates from Waltham Cross were resistant. In the Northern Paarl vineyards, 95% of the isolates from Dan-ben-Hannah and 95% of the isolates from Waltham Cross were resistant. The sensitivity to folpet of the isolates from the different populations, as determined by the mycelium growth sensitivity test, is given in Table 3. In the case of the iprodione-sensitive isolates from vineyards in Simondium, folpet EC_{50} values ranged from 4.9 to 29.1 µg/mL for the Dan-ben-Hannah and 15.0 to 43.5 µg/mL for the Waltham Cross sub-populations, respectively. Folpet EC_{50} values of the iprodione-resistant isolates, on the other hand, ranged from 19.7 to above 100 µg/mL for the for the Dan-ben-Hannah sub-population. The single iprodione-resistant isolate obtained from Waltham Cross displayed an EC_{50} value of 19.8 µg/mL. In the Northern Paarl sub-populations, where the isolates were predominantly iprodione-resistant, folpet EC_{50} values of the latter isolates ranged from 21.5 to above 100 µg/mL.

The comparative mycelium growth sensitivity of isolates to folpet, selected from different regional sub-populations that represented three iprodione sensitivity classes, is given in Table 4. The folpet EC_{50} values of the sensitive isolates ranged from 3.9 to 19.7 µg/mL, those of the ultra-low-level resistant isolates ranged from 5.5 to 62.1 µg/mL, and those of the low-level resistant isolates ranged from 11.3 to above 100 µg/mL.

According to a Pearson correlation, the EC_{50} values for mycelium growth and spore germination of 13 isolates correlated significantly (R=0.87666, P<0.0001). EC_{50} values obtained with spore germination tests were, however, markedly lower than those obtained for mycelium growth tests and ranged from 0.211 to 1.798 µg folpet/mL (Fig. 1). The spore germination EC_{50} values were grouped into two distinct groups, one consisting of 10 sensitive (average EC_{50} value 0.432 µg/mL) isolates and another consisting of four resistant (average EC_{50} value 1.532 µg/mL) isolates. The resistance value (EC_{50} value of resistant sub-population / EC_{50} value of sensitive sub-population) for spore germination was calculated at 3.55.

TABLE 2

Mean resistance frequency of *B. cinerea* isolates to iprodione obtained from Dan-ben-Hannah and Waltham Cross table grape vineyards in two different localities.

Location	Cultivar	Number of isolates tested	Iprodione resistance frequency (%)
Simondium	Dan-ben-Hannah	23	61
	Waltham Cross	5	20
Northern Paarl	Dan-ben-Hannah	22	95
	Waltham Cross	21	95

TABLE 3

Folpet EC_{50} values obtained from mycelium growth tests with iprodione-sensitive and -resistant *B. cinerea* isolates from Dan-ben-Hannah and Waltham Cross table grape vineyards in two different localities.

Location	Cultivar	Folpet EC_{50} values (µg a.i./mL)	
		Iprodione-sensitive	Iprodione-resistant
Simondium	Dan-ben-Hannah	4.9 – 29.1	19.7 – >100
	Waltham Cross	15.0 – 43.5	19.8
Northern Paarl	Dan-ben-Hannah	11.9	21.6 – >100
	Waltham Cross	15.6	21.5 – >100

TABLE 4

EC_{50} values for folpet and iprodione of *B. cinerea* isolates obtained from various vineyards in different viticultural regions.

Dicarboximide sensitivity class[a]	EC_{50} values (µg a.i./mL)	
	Vinclozolin	Folpet
Sensitive	0.001 – 0.3	3.9 – 19.7
Ultra-low-level resistant	0.8 – 1.8	5.5 – 62.1
Low-level resistant	2.1 – 5.1	11.3 – >100

[a]Isolates were selected from a culture collection consisting of *B. cinerea* isolates obtained from various South African table grape vineyards (Fourie & Holz, 1998).

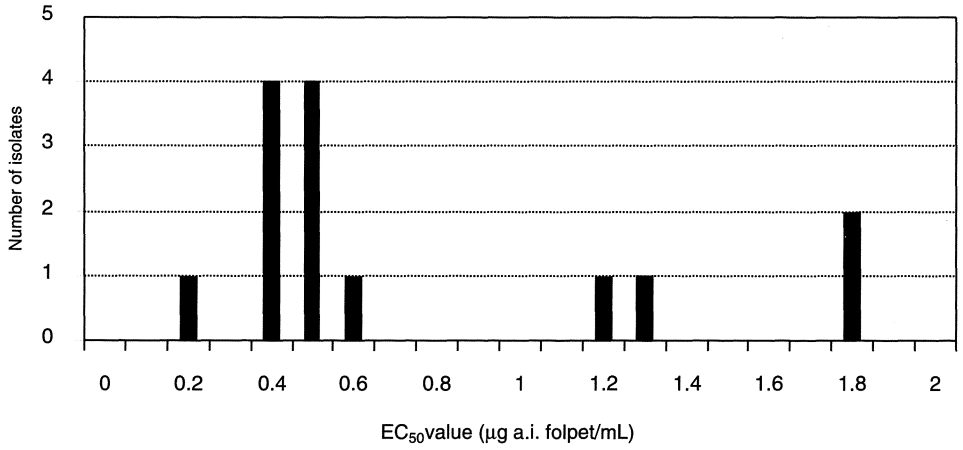

FIGURE 1

Distribution of folpet EC_{50} values for spore germination of *B. cinerea* isolates with EC_{50} values for mycelium growth ranging from 4.93 to >100 µg folpet/mL.

DISCUSSION

This study confirmed the resistance to iprodione in *B. cinerea* isolates obtained from table grape vineyards subjected to high-schedule DC and folpet programmes. According to Fourie and Holz (1998), a markedly higher DC resistance balance value (Beever *et al.*, 1991) prevailed in these vineyards compared to low DC-schedule vineyards. Mycelium growth and spore germination tests clearly indicated reduced sensitivity to folpet in the DC-resistant *B. cinerea* sub-populations obtained from vineyards exposed to high DC and folpet schedules. The sensitivity tests showed that folpet EC_{50} values of the isolates tended to increase with an increase in DC resistance, which confirmed earlier reports (Hunter *et al.*, 1987; Washington *et al.*, 1992; Raposo *et al.*, 1996) on cross-resistance between these fungicide groups. This finding is substantiated by the shift in folpet sensitivity displayed by the previously characterised (Fourie & Holz, 1998) ultra-low- and low-level DC-resistant *B. cinerea* isolates obtained from other regional sub-populations. The early increase of DC resistance frequencies in *B. cinerea* observed in vineyards under the high DC schedule (Fourie & Holz, 1998) can therefore be attributed to cross-resistance in sub-populations to DCs and folpet. The findings furthermore suggest that, by maintaining a high folpet and DC schedule programme, DC resistance frequencies in the Northern Paarl vineyards were kept at a high level. Compared to earlier fungicide programmes, no folpet and less DCs were applied in the Simondium vineyards and consequently lower DC-resistance frequencies were observed in these vineyards than those reported earlier (Fourie & Holz, 1998).

Reduced sensitivity in *B. cinerea* to both iprodione and folpet can be ascribed to the mode of action of these fungicides. Folpet and related compounds are reported to target the glutathione system (Siegel & Sisler, 1968a, 1968b; Barak & Edgington, 1984b, 1984c). Recent work by Ellner (1996) provides evidence of a possible dual mechanism of action of the DCs in *B. cinerea*: initiation of lipid peroxidation by the generation of reactive oxygen and the reduction of glutathione concentration by reducing equivalents and co-substrate of membrane-protecting and other glutathione-dependent enzymes. Ellner (1996) also noted that enhanced levels of glutathione synthetase with reduced sensitivity in resistant strains of *B. cinerea* might be a mechanism of resistance to the DC fungicides. Enhanced glutathione biosynthesis in *B. cinerea* was also discussed as a possible resistance mechanism to captab and chlorothalonil (Barak & Edgington, 1984b, 1984c). Folpet is a multi-site inhibitor in the sulphenimide group. The mode of resistance to these compounds is more complex than that of the single-site inhibitors, like the DCs. This phenomenon is substantiated by the wide range in folpet EC_{50} values displayed by the isolates used in this study. Given the similarities in mode of action, resistance in *B. cinerea* to DCs and folpet can therefore more specifically be characterised as incomplete cross-resistance, instead of using the broader term, multiple resistance.

Malathrakis (1989) recovered dichlofluanid-resistant *B. cinerea* isolates from greenhouse-cultivated vegetables that were also resistant to chlorothalonil and captab. The dichlofluanid-resistance value for spore germination of these isolates was 4.5. This resistance value for spore germination is comparable to the 3.6 found for the isolates that we investigated. Washington *et al.* (1992) reported similar EC_{50} values of *B. cinerea* for dichlofluanid to those found in this study for folpet, and also reported ineffective control of strawberry grey mould by dichlofluanid, due to resistance build-up. *Botrytis cinerea* isolates resistant to captan were stable and had similar pathogenicity to that of wild-type strains (Barak & Edgington, 1984a). Practical folpet resistance in *B. cinerea* in Western Cape vineyards was not reported and further studies are needed to determine the *in vivo* pathogenicity of strains with reduced sensitivity to folpet in the presence and absence of the fungicide.

The high potential for resistance increase in vineyards with high resistance balance values poses certain resistance management problems in high DC-schedule vineyards. A similar phenomenon was observed in New Zealand kiwi orchards where the early application of benomyl for the control of *Sclerotinia* blossom blight caused the DC resistance frequency to increase early in the season prior to the application of any DCs. Consequently, the efficacy of DCs that were applied later in the season was reduced, which resulted in increased post-harvest decay by *B. cinerea* (Pak, *et al.*, 1995). Our findings suggest that, due to reduced folpet sensitivity in DC-resistant *B. cinerea* strains, folpet applications would exert additional selection pressure on the DC-resistant sub-population. This phenomenon was not observed in the low and medium DC-schedule vineyards, despite the use of folpet during the pre-blossom stage (Fourie, 1996; Fourie & Holz, 1998). The repetitive use of this broad-spectrum fungicide in vineyards with a history of high grey mould incidence and a high DC-schedule and/or vineyards with high DC resistance balance values should thus be avoided.

LITERATURE CITED

Barak, E. & Edgington, L.V., 1984a. *Botrytis cinerea* resistant to captan: the effect of inoculum age and type on response to the fungicide. Can. J. Plant Pathol. 6, 211-214.

Barak, E. & Edgington, L.V., 1984b. Cross-resistance of *Botrytis cinerea* to captan, thiram, chlorothalonil, and related fungicides. Can. J. Plant Pathol. 6, 318-320.

Barak, E. & Edgington, L.V., 1984c. Glutathione synthesis in response to captan: a possible mechanism for resistance of *Botrytis cinerea* to the fungicide. Pestic. Biochem Physiol. 21, 412-416.

Beever, R.E., Pak, H.A. & Laracy, E.P., 1991. An hypothesis to account for the behaviour of dicarboximide-resistant strains of *Botrytis cinerea* in vineyards. Plant Pathol. 40, 342-346.

De Klerk, C.A., 1985. Chemical control programme for vine diseases and pests. Farming in South Africa. Viticultural and Oenological Series 200. Nietvoorbij Institute for Viticulture and Oenology, Private Bag X5026, 7599 Stellenbosch, Republic of South Africa.

Ellner, F.M., 1996. The glutathione system a novel target of dicarboximides in *Botrytis cinerea*. In: H. Lyr, P.E. Russel & H.D. Sisler (eds.) Modern Fungicides and Antifungal Compounds. Intercept Ltd. pp. 133-140.

Fourie, P.H., 1996. Dicarboximide resistance in *Botrytis cinerea* in South African table grape vineyards. M.Sc. thesis, Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.

Fourie, P.H. & Holz, G., 1998. Frequency of dicarboximide resistant strains of Botrytis cinerea in South African table grape vineyards and influence of spray schedules on resistant sub-populations. S. Afr. J. Enol. Vitic. 19, 3-9.

Hunter, T., Brent, K.J., Carter, G.A. & Hutcheon, J.A., 1987. Effects of fungicide spray regimes on incidence of dicarboximide resistance in grey mould (*Botrytis cinerea*) on strawberry plants. Ann. Appl. Biol. 110, 515-525.

Leroux, P. & Clerjeau, M., 1985. Resistance of *Botrytis cinerea* Pers. and Plasmopara viticola (Berk. & Curt.) Berl. and de Toni to fungicides in French vineyards. Crop Protection 4, 137-160.

Leroux, P. & Fritz, R., 1984. Antifungal activity of dicarboximides and aromatic hydrocarbons and resistance to these fungicides. In: Trinci, A.P.J. & Ryley, J.F. (eds.) Mode of action of antifungal agents. Cambridge University Press. pp. 207-237.

Löcher, F.J. & Lorenz, G., 1991. Methods for monitoring the sensitivity of *Botrytis cinerea* to dicarboximide fungicides. pp. 341-345 in FRAC Methods for Monitoring Fungicide Resistance. EPPO Bulletin 21, 291-355.

Malathrakis, N.E., 1989. Resistance of *Botrytis cinerea* to dichlofluanid in greenhouse vegetables. Plant Dis. 73, 138-141.

Nel, A., Krause, M., Ramautar, N. & Van Zyl, K., 1999. A Guide for the Control of Plant Diseases. Directorate: Agricultural Production Input, National Department of Agriculture, Republic of South Africa.

Pak. H.A., Manning, M.A. & Pennycook, S.R., 1995. Effect of early season fungicides on resistance dynamics of *Botrytis cinerea* populations in New Zealand kiwifruit orchards. In: Scientific Programme and Abstracts of 10th Biennial Australasian Plant Pathology Society Conference, Lincoln University, New Zealand (28-30 August 1995), p. 55.

Pommer, E.-H. & Lorenz, G., 1982. Resistance of *Botrytis cinerea* Pers. To dicarboximide fungicides – a literature review. Crop Protection 1, 221-230.

Pommer, E.-H. & Lorenz, G., 1995. Dicarboximide fungicides. In: H. Lyr (ed.) Modern selective fungicides. Gustav Fischer. pp. 99-118.

Raposo, R., Delcan, J., Gomez, V. & Melgarejo, P., 1996. Distribution and fitness of isolates of *Botrytis cinerea* with multiple fungicide resistance in Spanish greenhouses. Plant Pathol. 45, 497-505.

Siegel, M.R. & Sisler, H.D., 1968a. Fate of the phthalimide and trichloromethylthio ($SCCl_3$) moieties of folpet in toxic action on cells of *Saccharomyces pastorianus*. Phytopathol. 58, 1123-1129.

Siegel, M.R. & Sisler, H.D., 1968b. Reactions of folpet with purified enzymes, nucleic acids and subcellular components of *Saccharomyces pastorianus*. Phytopathol. 58, 1129-1133.

Snedecor, G.W. & Cochran, W.G., 1967. Correlation. In: Statistical Methods 6th Ed. Iowa State University Press, Ames, Iowa, USA. pp. 172-198.

Van der Merwe, G.G., Geldenhuys, P.D. & Botes, W.S., 1991. Guidelines for the preparation of table grape cultivars for export. Unifruco, Parc du Cap, PO Box 505, Bellville 7535, South Africa.

Vermeulen, A.K., 1999. A Guide to the Use of Registered Fungicides and Pesticides against Grapevine Diseases and Pests: Wine grapes. ARC – Fruit, Vine and Wine Research Institute, Private Bag X5026, Stellenbosch, 7599, South Africa.

Ward, J.H., 1963. Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association 58, 236-244.

Washington, W.S., Shanmuganathan, N. & Forbes, C., 1992. Fungicide control of strawberry fruit rots, and the field occurrence of resistance of *Botrytis cinerea* to iprodione, benomyl and dichlofluanid. Crop Protection 11, 355-360.