Metalaxyl Sensitivity Status of Downy Mildew Populations in Western Cape Vineyards

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Grapevine downy mildew, caused by *Plasmopara viticola*, is a sporadic, but destructive disease of grapevines in South Africa. Despite extensive fungicide spray programmes, disease control during disease-conducive weather periods is often inadequate, and severe losses were experienced during recent epidemics. Poor disease control can be attributed to several factors, one of which may be reduced efficacy of fungicides due to resistance build-up in the pathogen populations. *P. viticola* isolates were sampled during the 1999/2000, 2000/2001 and 2001/2002 seasons from various vineyards in the Western Cape province of South Africa and screened for sensitivity to metalaxyl using the floating leaf-disc method recommended by the Fungicide Resistance Action Committee. Minimum inhibitory concentration (MIC) values for 813 isolates were determined. Only 5.8% of the isolates tested had MIC values of 10 µg/mL and lower. The majority of the isolates (94.2%) showed MIC values of 100 µg/mL or more. These isolates were designated resistant to highly resistant. With the exception of one vineyard in a remote area where phenylamides had not previously been used, very high resistance frequencies were observed, irrespective of whether the samples were taken early or late in the season. This observation, supported by the consistent high resistance frequencies recorded over time in individual vineyards, is indicative of the stability of metalaxyl resistance in grapevine downy mildew populations. In view of the widespread occurrence and stability of resistance against metalaxyl, the use of phenylamide formulations for the control of downy mildew in Western Cape vineyards should be reconsidered.

Downy mildew, caused by the oomycete fungus *Plasmopara viticola* (Berk. & Curt.) Berlese & de Toni), is a sporadic, but destructive disease of grapevines in the Western Cape province of South Africa (Marais, 1973; Fourie & Vermeulen, 2000, 2002). Despite several cultural practices that can be implemented to manage the disease, control is mainly by means of fungicide sprays. According to Schwinn and Staub (1995), about 20% of the world fungicide market in 1991 was devoted to the control of diseases caused by oomycete pathogens, of which 34% were specifically for the control of grapevine downy mildew.

In the late 1970s, Urech *et al.* (1977) reported on a novel fungicide for the control of late blight, downy mildews and related soilborne diseases. This fungicide, metalaxyl, which is in the phenylamide group, was highly fungitoxic and showed protective as well as curative activity against all *Peronosporales*. Phenylamides have a highly specific mode of action, which is the inhibition of ribosomal RNA synthesis, thus resulting in fungal growth inhibition (Davidse, 1995). It is furthermore rapidly taken up in plant tissue and showed high acropetal systemicity, thus providing protection of new growth (Schwinn & Staub, 1995). Given these attributes, metalaxyl was found to be very effective against grapevine downy mildew and was widely and exclusively used. Due to the specific mode of action, a single change in the metalaxyl target site of fungal cells constituted high levels of fungicide resistance (Davidse, 1995). Consequently resistance to metalaxyl developed two years after its market introduction due to intensive, continuous and exclusive use (Morton & Urech, 1988). Cross-resistance was also reported in *P. viticola* between the different phenylamide fungicides (Diriwächter *et al.*, 1987).

Several phenylamide products are currently registered for control of grapevine downy mildew in South Africa (Nel *et al.*, 1999) and are being used by grape growers in the Western Cape province. Despite extensive fungicide spray programmes with these and other products, disease control during disease-conducive weather periods is often inadequate, and severe losses were experienced during recent epidemics (Fourie & Vermeulen, 2000, 2002). Poor disease control can be attributed to several reasons, one of which may be reduced efficacy of fungicides due to resistance build-up in the pathogen populations. No previous study was conducted locally on phenylamide resistance development in grapevine downy mildew. The aim of this study was therefore to determine the sensitivity status of downy mildew populations in Western Cape vineyards to the most commonly used phenylamide fungicide, metalaxyl.

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MATERIALS AND METHODS

Sample collection

Grapevine leaves with downy mildew symptoms were collected throughout the 1999/2000, 2000/2001 and 2001/2002 growing seasons from various vineyards in the Western Cape province. At least 30 leaf samples were taken from each vineyard and placed in individual plastic bags. Samples were kept in cool boxes and immediately transported to the laboratory. Sterile paper towels, moistened with sterile, deionised water, were added to each plastic bag. The moistened bags, each containing a downy mildew symptomatic leaf, were incubated for 3 days at 22°C in the dark to stimulate sporulation of the fungus (Stähle-Csech *et al.*, 1992). Sporangia were collected from one individual lesion per leaf sample and this inoculum was used for testing of sensitivity to metalaxyl. Through genetic analysis of single downy mildew lesions, Gobbin *et al.* (2003) have shown that a lesion was in most cases (97.8%) caused by a single genotype of *P. viticola*. The use of single-lesion isolates (Genet *et al.*, 1997) was therefore warranted and allowed for quantitative assessment of data.

Test plants

A continuous supply of young, fresh grapevine leaves for use in sensitivity tests was needed. Three hundred Chardonnay (clone CY3-XI2) vines, grafted on Richter 99 (clone RY179-BXP1), were obtained from a commercial grapevine nursery. These vines were planted in a sand/bark mixture in 5 L plastic nursery bags and grown in a glasshouse at 25°C. No chemicals were sprayed on the foliage. Plants were kept free of powdery mildew by the vapour action from two 10 x 30 cm cotton cloths that were dipped in formulation-strength penconazole (Topaz®, EW 200 g/L, Syngenta). Mealy bugs were controlled by annual root applications of imidacloprid (Confidor®, SC 350 g/L, Bayer). The third and fourth leaves from the tips of growing shoots were harvested and used in sensitivity tests to ensure uniformity in age and susceptibility of leaves to infection by downy mildew spores.

Leaf disc assay

Leaf discs, cut from healthy Chardonnay leaves, were floated upside down on 20 mL of a specific concentration of the metalaxyl (Syngenta technical grade, 98% pure) as described in the Fungicide Resistance Action Committee (FRAC) methods (Stähle-Csech *et al.*, 1992). Concentrations used, were 0 (untreated control), 0.1, 1.0, 10, 100 and 200 µg/L. Leaf discs (10 discs per fungicide concentration) were inoculated with one 10 µL droplet per disc from a sporangiospore suspension of the fungus. After 7 days' incubation at 22°C, the fungal growth was evaluated on a scale of 0 – 4 (0 – no fungal growth; 1 – very small lesion without sporulation; 2 – sporulating lesion smaller than droplet; 3 – sporulating lesion the same size as the original droplet; 4 – sporulating lesion exceeding the size of the droplet). Minimum inhibitory concentrations [MIC values; lowest concentration where sporulation (rating of 2 to 4) was not observed; Gullino *et al.*, 1997; Genet & Vincent, 1999] were determined from the raw data for each isolate. Data from any given isolate were discarded if fewer than seven untreated leaf discs (control) yielded sporulating lesions. Isolates with the same MIC values were grouped and resistance factors (RF = MIC value of field isolate / MIC value of sensitive reference isolate; Stähle-Csech *et al.*, 1992) of each group calculated. Resistance frequencies (number of resistant isolates as a percentage of the total number of isolates tested; Fourie & Holz, 1998) were furthermore calculated for each vineyard tested.

RESULTS AND DISCUSSION

MIC values for 813 isolates were determined and their distribution is given in Table 1. The most sensitive group (MIC value of 0.1 µg/mL) was regarded as the sensitive reference group, and the MIC value from this group was used to determine the resistance factors of the other groups. Given the widespread use of phenylamides, migration of resistant inoculum (Genet & Vincent, 1999), as well as the wet-weather dependence and obligate nature of *P. viticola*, a sensitive reference isolate could not be obtained and was therefore not included in the screenings. However, during the 1999/2000 season, 5 isolates were obtained from a Riesling vineyard in Bonnievale, where phenylamides had never been used (Table 2). Given the predominantly drier climate of this area, downy mildew occurs infrequently. All these isolates tested sensitive to metalaxyl with MIC values of 0.1 µg/mL. Consequently, the use of the MIC value of the most sensitive group (0.1 µg/mL) as reference was justified. According to Stähle-Csech *et al.* (1992), resistance factors for phenylamides are in most cases higher than 100. Hence, the isolates with MIC values of 1 µg/mL (RF = 10) were characterised as "sensitive", whereas isolates with MIC values of 10 µg/mL (RF = 100) were characterised as "low-level resistant", those with MIC values of 100 µg/mL (RF = 1000) as "resistant", and those with MIC values of 200 µg/mL (RF = 2000) and MIC values of >200 µg/mL (RF = >2000) as "highly resistant". Only 5.8% of the isolates tested were designated as low-level resistant or sensitive. The majority of the isolates (94.2%) were designated resistant to highly resistant. These results clearly show the high level and frequency of resistance against metalaxyl in downy mildew populations in Western Cape vineyards.

Given the uncertainty regarding the practical implications of resistance factors, low-level resistant isolates were not regarded as "resistant" when resistance frequencies for the various vineyards were determined. Resistance frequencies for all vineyards monitored are given in Table 2. With the exception of the Bonnievale Riesling vineyard, very high resistance frequencies were observed in the various regions and cultivars monitored, irrespective of whether the samples were taken early or late in the

TABLE 1

Distribution of MIC values for metalaxyl of the 813 downy mildew isolates tested during the 1999/2000, 2000/2001 and 2001/2002 seasons.

MIC value	Number of isolates	Resistance factor[x]	Characterisation
0.1	21	–	Sensitive
1	13	10	Sensitive
10	13	100	Low-level resistant
100	143	1000	Resistant
200	149	2000	Highly resistant
>200	474	>2000	Highly resistant

[x]Resistance factor = MIC value of field isolate / MIC value of sensitive reference group (0.1 µg/mL).

TABLE 2

Frequency of metalaxyl-resistant downy mildew isolates in vineyards monitored during the 1999/2000, 2000/2001 and 2001/2002 seasons.

Date sampled	Farm code	Region	Cultivar	Isolates tested	R%[x]	Phenylamide history
1999/2000 season						
11 Nov 99	Nvb	Stellenbosch	Bukettraube	1	100	Yes
11 Nov 99	GBS	Wellington	Festival Seedless[y]	6	100	Yes
11 Nov 99	GBS	Wellington	Sultana Seedless	9	77.8	Yes
10 Dec 99	Sfield	Robertson	Cape Riesling	2	50	?
10 Dec 99	Jbg	Stellenbosch	Chardonnay	3	100	Yes
10 Dec 99	Bon	Bonnievale	Cape Riesling	5	0	No
10 Dec 99	Dhm	Stellenbosch	Muscat d' Alexandrie	1	100	?
2000/2001 season						
12 Oct 00	KWV	Paarl	Chardonnay	2	66.7	Yes
12 Oct 00	GBS	Wellington	Festival Seedless[y]	13	76.9	Yes
23 Oct 00	ER	Paarl	Bien Donné	24	95.8	Yes
7 Nov 00	DK	Northern Paarl	Victoria	21	95.2	Yes
29 Nov 00	BB	Simondium	Chardonnay	5	100	No
29 Nov 00	LL	Stellenbosch	Bukettraube	2	100	?
6 Dec 00	WGV	Stellenbosch	Chardonnay[y]	16	93.8	Yes
31 Jan 01	DK	Northern Paarl	Festival Seedless	26	100	Yes
8 Feb 01	GBS	Wellington	Sunred Seedless	19	94.7	Yes
23 Feb 01	NVB	Stellenbosch	La Rochelle	17	94.1	No
16 Mar 01	JF	Malmesbury	Various	20	95	No
2001/2002 season						
24 Oct 01	R-V	Stellenbosch	Cabernet Sauvignon	29	89.7	No
31 Oct 01	GBS	Wellington	Festival Seedless[y]	16	81.3	Yes
8 Nov 01	WGV	Stellenbosch	Chardonnay[y]	28	100	Yes
8 Nov 01	Olyv	Agter-Paarl	Sunred Seedless	2	50.0	?
13 Nov 01	GBS	Wellington	Festival Seedless[y]	23	100	Yes
13 Nov 01	GBS	Wellington	Sunred Seedless	26	92.3	Yes
20 Nov 01	AdT	Helderberg	Cabernet Sauvignon	30	96.7	Yes
20 Nov 01	Pik	Helderberg	Chardonnay	28	100	No
28 Nov 01	Zet	Stellenbosch	Cabernet Sauvignon	27	92.6	Yes
5 Dec 01	Vlok	Riebeeck-Kasteel	Regal Seedless	15	93.3	Yes
10 Dec 01	Vdal	Vredendal	Chenin blanc	29	100	?
13 Dec 01	Khof	Stellenbosch	Villard blanc	24	95.8	Yes
13 Dec 01	NVB	Stellenbosch	Riesling	23	100	No
23 Jan 02	DWt	Wellington	Red Globe	23	95.7	Yes
23 Jan 02	vSw	Wellington	Colombar	30	100	Yes
29 Jan 02	Lrac	Stellenbosch	Chardonnay	28	100	?
1 Feb 02	Bvue	Paarl	Waltham Cross	22	95.5	Yes
1 Feb 02	Grob	Stellenbosch	Pinotage	30	100	?
12 Feb 02	PN	Helderberg	Cabernet Sauvignon	10	100	No
20 Feb 02	Stuin	Stellenbosch	Cape Riesling	5	100	Yes
5 Mar 02	Nhof	Stellenbosch	Shiraz	24	100	No
6 Mar 02	Herm	Hermon	La Rochelle	27	88.9	?
13 Mar 02	Thel	Stellenbosch	Cabernet Sauvignon	15	60.0	Yes
14 Mar 02	GBS	Wellington	Sunred Seedless[y]	24	83.3	Yes
14 Mar 02	GBS	Wellington	Festival Seedless[y]	29	100	Yes
27 Mar 02	JDK	Paarl	Dan-ben-Hannah	25	100	Yes
27 Mar 02	EdR	Paarl	Waltham Cross	28	100	Yes

[x]R% = Resistance frequency (number of resistant isolates [excluding low-level resistant isolates] / total number of isolates tested).

[y]These vineyards were screened twice or more.

season. This observation, supported by the consistent high-resistance frequencies over time from the GBS Festival Seedless and Sunred Seedless vineyards, (76.9% to 100% and 83.3% to 94.7%, respectively), is indicative of the stability of metalaxyl resistance in grapevine downy mildew. Phenylamides have furthermore not been applied in these vineyards for several years (results not shown). High frequencies of metalaxyl resistance in vineyards that had no history of phenylamide usage might also be an indication of long-range aerial dispersal of sporangia from sporulating downy mildew lesions. Genet and Vincent (1999) also suggested that mixing of populations occurs over relatively large distances. This observation holds serious implications for the management of fungicide resistance in grapevine downy mildew.

This survey represents the first study on phenylamide sensitivity in grapevine downy mildew populations in the Western Cape province of South Africa. Previous studies regarding phenylamide resistance in South Africa involved the potato and tomato late blight fungus, *Phytophthora infestans* (McLeod *et al.*, 2001). Resistance development was localised to certain areas, but was shown to be stable in the absence of phenylamide selection pressure. In the present study, metalaxyl resistance appeared to be stable and high frequencies were observed in vineyards where phenylamides have not been used for several years, indicating that resistant *P. viticola* isolates do not suffer from reduced fitness. This phenomenon negates the possible management of phenylamide resistance through the alternation with non-related chemistry, as is recommended by FRAC *(www.gcpf.org/frac)*. Given the widespread occurrence of high levels and frequencies of resistance against metalaxyl found, and the reported cross-resistance between the phenylamide compounds (Diriwächter *et al.*, 1987), the use of phenylamide formulations for the control of grapevine downy mildew should be reconsidered in Western Cape vineyards. Field trials should, however, be performed to ascertain whether the monitored resistance levels constitute practical resistance.

LITERATURE CITED

Davidse, L.C., 1995. Phenylamide fungicides – biochemical action and resistance. In: Lyr, H. (ed.). Modern Selective Fungicides. Gustav Fischer Verlag, New York. pp. 347-354.

Diriwächter, G., Sozzi, D., Ney, C. & Staub, T., 1987. Cross-resistance in *Phytophthora infestans* and *Plasmopara viticola* against different phenylamides and unrelated fungicides. Crop Protection 6, 250-255.

Genet, J.L., Steva, H., Vincent, O. & Cazenave, C., 1997. A method for measuring the level of sensitivity of *Plasmopara viticola* populations to cymoxanil. EPPO Bull. 27, 217-225.

Genet, J.L. & Vincent, O., 1999. Sensitivity of European *Plasmopara viticola* populations to cymoxanil. Pestic. Sci. 55, 129-136.

Gullino, M.L., Mescalchin, E. & Mezzalama, M., 1997. Sensitivity to cymoxanil in populations of *Plasmopara viticola* in northern Italy. Plant Pathol. 46, 729-736.

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.

Fourie, P.H. & Vermeulen, A.K., 2000. Donsige skimmel: 'n sporadiese, dog vernietigende siekte van wingerd. Wynboer 134, 11-14.

Fourie, P.H. & Vermeulen, A.K., 2002. Nabetragting van die 2001/2002 donsige skimmel epidemie. Wynboer 158, 8-10.

Gobbin, D., Pertot, I. & Gessler, C., 2003. Identification of microsatellite markers for *Plasmopara viticola* and establishment of high throughput method for SSR analysis. Eur. J. Plant Path. 109, 153-164.

Marais, P.G., 1973. Die biologie en epidemiologie van donsskimmel in Wes-Kaapland. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

McLeod, A., Denman, S., Sadie, A. & Denner, F.D.N., 2001. Characterization of South African isolates of *Phytophthora infestans*. Plant Dis. 85, 287-291.

Morton, H.V. & Urech, P.A., 1988. History of the development of resistance to phenylamide fungicides. In: Delp, C.S. (ed.). Fungicide Resistance in North America. APS Press, St. Paul. pp. 59-60.

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.

Schwinn, F. & Staub, T., 1995. Phenylamides and other fungicides against Oomycetes. In: Lyr, H. (ed.). Modern Selective Fungicides. Gustav Fischer Verlag, New York. pp. 323-346.

Stähle-Csech, U., Gisi, U. & Sozzi, D., 1992. Determination of the sensitivity of *Plasmopara viticola* to phenylamides. EPPO Bull. 22, 314-316.

Urech, P.A., Schwinn, F. & Staub, T., 1977. CGA 48988, a novel fungicide for the control of late blight, downy mildews and related soil borne diseases. Proc. 9th Brit. Crop. Prot. Conf. pp. 623-631.