Resistance in *Uncinula necator* to Triazole Fungicides in South African Grapevines

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Submitted for publication: March 2000
Accepted for publication: May 2000
Key words: Fungicide resistance, grape powdery mildew, *Oidium tuckeri*

The distribution of *Uncinula necator* variants resistant to triadimenol, penconazole and flusilazole were determined in vineyards with suspected resistance in the regions Tulbagh, Franschhoek, Stellenbosch, De Doorns, Riebeeck Kasteel and Paarl. The regional subpopulations had all been exposed to triadimefon or triadimenol prior to 1989, when these fungicides were phased out and other demethylation-inhibiting fungicides (DMIs) were applied. The occurrence of resistant variants in the subpopulations was compared with those in a vineyard in the Ceres Karoo region, which was isolated by two mountain ranges from the viticultural regions and where triadimefon was used prior to 1989, then abandoned. No other DMIs were applied. A discriminatory germ tube length was used as a criterion to distinguish between sensitive and tolerant conidia at a discriminatory fungicide dose of 0.3 µg/mL. All the populations showed reduced sensitivity to triadimenol. This finding indicated an earlier shift in triadimenol sensitivity in the subpopulations and showed that resistant variants are sufficiently competitive to become established in vineyards. Cross-resistance between the triazoles was indicated by the frequency at which resistant variants occurred in subpopulations. The Ceres Karoo population was at baseline sensitivity level for penconazole and flusilazole. However, the four populations (De Doorns, Franschhoek, Riebeeck Kasteel and Stellenbosch) which showed the highest shifts in sensitivity to triadimenol, also displayed a high level of reduced sensitivity to flusilazole. This was in spite of the fact that only the Stellenbosch population was regularly treated with flusilazole. The other three populations were predominantly exposed to penconazole. Reduced sensitivity to penconazole was furthermore most prevalent in the Paarl K, Paarl I, Riebeeck Kasteel and De Doorns populations. Of these populations, Paarl K and Paarl I received predominantly penconazole, whereas the other two populations were treated with a range of DMIs. Penconazole ED_{50} values for the Paarl K, Paarl I, Riebeeck Kasteel and De Doorns pathogen populations (which showed the highest shifts in sensitivity to this fungicide) were 0.908, 1.022, 1.253 and 1.942 µg/mL, respectively. In these populations, 53%, 38%, 71% and 91% of the conidia respectively belonged to the 1.0–3.0 µg/mL and higher resistant classes. Reduced sensitivity to flusilazole was most prevalent in the Stellenbosch, De Doorns, Riebeeck Kasteel and Franschhoek populations. Flusilazole ED_{50} values for these populations were 1.580, 1.813, 2.143, 3.885 µg/mL, respectively, whereas 83%, 82%, 96% and 79% of the conidia respectively belonged to the 1.0–3.0 µg/mL and higher resistant classes. These findings suggest a differing sensitivity of the pathogen to the three triazole fungicides which indicate that resistance to DMIs is a multigenic trait in *U. necator.*

Powdery mildew, caused by *Uncinula necator* (Schw.) Burr., is one of the most important diseases on grapevine worldwide (Pearson & Gadoury, 1992). Since the late 1970s, sulphur and dinocap have been supplemented with the demethylation-inhibiting fungicides to control the disease (Steva & Clerjeau, 1990; Gubler *et al.,* 1996). The demethylation-inhibiting fungicides, known collectively as DMIs, affect the ergosterol biosynthesis pathway of many fungi by a single-mode action (Scheinpflug & Kuck, 1987). This mode of action has led to concern about development of resistance to DMIs and the likelihood of cross-resistance among individual compounds in the group (Steva & Clerjeau, 1990). Recent studies showed that cross-resistance does exist among the DMIs used to control *U. necator* (Steva & Clerjeau, 1990; Erickson & Wilcox, 1997 Ypema *et al,* 1997). A time course study performed in one vineyard, where resistance was reported, demonstrated a steady increase in means of EC_{50} values for triadimefon, myclobutanil and fenarimol, even though triadimefon was the only fungicide applied. This suggests that selection pressures by triadimefon can influence resistance to myclobutanil and fenarimol as well, indicating that the genetic mechanisms conferring resistance to DMI fungicides are generally correlated (Gubler *et al,* 1996). However, the degree of cross-resistance might vary substantially between the DMIs. A substantially greater degree of cross-resistance exists between triadimenol (triazole) and myclobutanil (triazole) than between either of these fungicides and fenarimol (pyrimidine) (Erickson & Wilcox, 1997; Ypema *et al.,* 1997). DMI cross-resistance seems to be much more restricted in *U. necator* than in *Venturia inaequalis,* where generally high levels of cross-resistance do occur (Köller *et al.,* 1997). In the light of this it is possible that resistance to DMIs is a multigenic trait in *U. necator* and that one or more resistance genes are independent with respect to individual DMIs or groupings of them (Erickson & Wilcox; 1997, Ypema *et al.,* 1997).

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The distribution of sensitivities to a DMI fungicide among individuals in an *U. necator* population unexposed to the chemical is continuous, ranging from highly sensitive to less sensitive phenotypes (Erickson & Wilcox, 1997). The use of DMIs stimulates the selection process for isolates with elevated resistance levels and contributes to an increase in resistant variants in the *U. necator* population (Gubler *et al.,* 1996). This type of resistance is called polygenic resistance, where many mutant genes are required, each responsible for only a minor resistance step to achieve the highest level of resistance possible (Dekker, 1993). The various combinations of resistance genes result in a continuous distribution of sensitivities, so that distinct subpopulations cannot easily be recognised and it may be several years before resistance problems will arise in practice. This will depend on the fitness of resistance strains and the selection pressure by the fungicide (Dekker, 1993). This suggests that resistance to DMIs develops as a series of slow genetic shifts in the population. After triadimefon had been used for some years, losses in its efficacy were noted in 1984 in Portugal (Steva & Clerjeau, 1990), 1985 in California (Ouimette & Gubler, 1990), 1989 in New York (Pearson, 1990) and 1996 in Austria (Redl & Steinkellner, 1996). Resistance to triadimenol was reported during 1988 in Portugal and 1989 in France (Steva & Clerjeau, 1990), and in 1997 in New York (Erickson & Wilcox, 1997). Recently resistance has also been reported for myclobutanil and fenarimol (Gubler *et al,* 1996; Erickson & Wilcox, 1997), and for penconazole and pyrifenox (Redl & Steinkellner, 1996).

In South Africa the triazole fungicides triadimefon, triadimenol, penconazole, flusilazole, hexaconazole, myclobutanil, tebuconazole and fluquinconazole, the pyrimidines nuarimol and fenarimol, and the pyridine pyrifenox are registered for use on grapevines (Nel *et al.,* 1999). Extensive usage (4–9 applications per season) of these fungicides (De Klerk, 1988) and the fact that the disease occurs annually on grapevines locally (JHS Ferreira, ARC Fruit, Vine and Wine Research Institute, Private Bag X5026, Stellenbosch, 7599; personal communication), have stimulated the selection process for isolates with elevated resistance levels. Recently there have been several reports from producers of poor performance of DMI fungicides against *U. necator* on grapevines. Little is known about the presence of resistant variants of the pathogen and the possible occurrence of practical resistance to specific DMIs in *U. necator* populations in the viticultural regions. The aim of this study was to determine at what frequency variants resistant to the most commonly used fungicides exist in problem vineyards, and to determine the concentration of fungicide required to produce a given mortality of the pathogen population in problem vineyards relative to a population unexposed to the fungicide.

MATERIALS AND METHODS

Survey of fungicides and vineyards in viticultural regions: A survey was conducted (a) to determine which fungicides are the most commonly used for powdery mildew control on grapevine in the viticultural regions, (b) to identify vineyards with a proven history of non-exposure to DMIs from which populations at baseline sensitivity could be obtained, and (c) to identify vineyards with suspected practical resistance to DMI fungicides. This entailed sending a detailed questionnaire to extension officers, consultants and producers in the viticultural regions, visiting producers and examining vineyards. Based on the outcome of the survey, three fungicides (Table 1) were selected for use in the studies, namely penconazole (Topaz, 200 EW, Novartis, high usage), triadimenol (Bayfidan, 250 EC, Bayer, low usage) and flusilazole (Olymp, 100 EC, Agricura, moderate usage). Vineyards with different histories of DMI application (Table 2) and suspected practical resistance were selected in each of the regions Tulbagh, Franschhoek, Stellenbosch, De Doorns, Riebeeck Kasteel and Paarl.

Powdery mildew isolates: During 1996 to 1998 symptomatic bunches were collected from the different vineyards. On each farm approximately 50 bunches were cut at random from at least 30 rows per vineyard. The bunches were wrapped in clean healthy grapevine leaves, wrapped in newspaper and were used either on the day of collection or left overnight in a growth room (21°C ± 1) to allow further sporulation.

Fungicide sensitivity: The sensitivity of the different *U. necator* populations to the selected fungicides was evaluated on grape leaf disks (cultivar Cinsaut) according to the quantitative mycelium growth test method of Steva (1994). Cuttings obtained during July and August were left overnight in a captab (500 WP) solution before cold storage (4°C) in moist perlite in plastic bags. When needed, cuttings were removed from the plastic bags and placed in a water bath at 50°C for 30 minutes (Goussard & Orffer, 1979). Thereafter the cuttings were placed in 10 cm plastic pots filled with 2 cm of water and left in a growth room (27 – 30°C and 24 h light) to initiate budbreak and root formation. At root formation the cuttings were planted in 9 cm plastic pots filled with a 4:1 mixture of peat and vermiculite and placed in a greenhouse kept at 22–30°C and a photoperiod regulated by natural sunlight. The cuttings were watered with a 3:1:6 (46) Chemicult plant fertiliser compound with trace elements (1 g/L water) once a week. The cuttings were maintained free from powdery mildew using penconazole vapours (Szkolnik, 1983). Only the third fully expanded leaves of cuttings were used. Fine, shiny leaves which were of sufficient size for preparing 7 to 10 disks 18 mm in diameter were selected, surface sterilised for 3 min in 30% sodium hypochlorite and rinsed for 5 min in sterile deionised water. The leaves were dried between sterile filter paper sheets and leaf disks were cut with a corkborer.

TABLE 1

Survey of DMI fungicides sold for the control of powdery mildew on grapevine during 1995 in the Western Cape province.

Fungicide	Percentage of tonnage sold
Triazoles	
Penconazole	60
Tebuconazole/Triadimenol	10
Myclobutanil	8
Hexaconazole	5
Flusilazole	3
Triadimefon	1
Pyridines	
Pyrifenox	9
Pyrimidines	
Fenarimol	1
Other	3

TABLE 2

DMI fungicides most commonly used for powdery mildew control during the last 10 years in vineyards investigated in this study.

Region	Cultivars	Fungicides applied[a]	Applications	Disease history
Ceres Karoo	Thompson Seedless	None	0	Unknown. Low incidence at time of sampling. Only on leaves.
Tulbagh	Pinot Noir	Hexaconazole (0–3), penconazole (0–3), pyrifenox (0–2)	3–4	Increase over the last 3 seasons with high incidence during the 1997/1998 season. Leaves and berries.
Stellenbosch	Bukettraube	Flusilazole (3), myclobutanil/dinocap (0–1)	3–4	Constant high incidence. Leaves and berries.
Franschhoek	Chardonnay	Penconazole (0–5), pyrifenox (0–9)[b], hexaconazole (0–4)	3–9	Increase over the last 4 seasons. Leaves and berries.
Paarl P[c]	Dauphine	Hexaconazole (0–2), penconazole (0–8)	2–8	High. Vineyard neglected since 1995. Only 2 triazole applications late in the season. Leaves and berries.
Paarl K	Dan-ben-hannah	Penconazole (1–7), myclobutanil/dinocap (0–8)	7–9	High. On rachii and pedicels later in the season.
Paarl I	Dauphine	Penconazole (0–7), pyrifenox (0–1), myclobutanil/dinocap (0–5)	7	High. On rachii and pedicels later in the season.
De Doorns	Dauphine	Penconazole (0–7), flusilazole (0–7), nuarimol (0–2)	7	High. On rachii and pedicels later in the season.
Riebeeck Kasteel	Dauphine	Penconazole (0–7), myclobutanil (0–5), flusilazole (0–5), myclobutanil/dinocap (0–5), fenarimol (0–1)	7	Increase over the last 4 seasons, especially on rachii and pedicels later in the season.

[a] Numbers in parenthesis give the fungicide application range per season.

[b] Pyrifenox has been applied only since the 1995/1996 season.

[c] Annual number of fungicide applications inconsistent.

A stock solution of 5000 mg a.i./L was prepared for each fungicide in a 100 mL glass container. A range of fungicide concentrations was then prepared from the stock solution. The upper surface of the leaf disks was placed on sterile filter paper saturated with 3 mL of fungicide in Petri dishes and incubated at 21°C ± 1°C. Ten disks (each from a different leaf) were used for each fungicide concentration plus 10 water treated disks for each fungicide. Disks in the untreated control were treated with sterile deionised water. After 24 h the disks were removed from the Petri dishes, turned over and allowed to dry for 10 min in a laminar flow cabinet. The disks were then placed in Petri dishes containing water agar medium (20g/L) supplemented with benzimidazole (30mg/L).

For inoculation, conidia were successively blown from the infected bunches into the top of a settling tower with a pressure pump using the method of Steva (1992). The conidia were allowed to settle on the upper surface of the leaf disks in the Petri dishes which were positioned on the floor of the settling tower. The dimensions of the Plexiglass tower were 120 x 35 x 40 cm (height x depth x width). Inoculum density, distribution (± 1000 conidia/cm^2) and viability were determined using glass-slides placed at the bottom of the settling tower. After inoculation, the Petri dishes were closed and placed in sealed, clear plastic boxes (9 x 30 x 20 cm), the bottoms of which were lined with moist paper towels. Dishes with leaf disks treated with one fungicide at one concentration were confined to one plastic box to avoid the effects of vapour action of the fungicides. The dishes were incubated in a growth chamber at 21°C ± 1 with 16 h light per day.

Germinated conidia were removed after 72 h by touching the upper surface of the leaf disks with adhesive tape, which was then stained in a drop of cotton blue on a microscope slide. Germ tube length of 30 conidia per leaf disk (5–10 disks per concentration) were measured using a microscope (100X). Mean germ tube length were used as the criterion to distinguish between conidia sensitive and resistant to the fungicide. Conidia yielding germ tubes shorter than the discriminatory length, determined by preliminary studies for each population, were considered sensitive, and those yielding germ tubes longer than the discriminatory length, were considered resistant to a given fungicide at a specific concentration. The frequency variants belonging to a given fungicide sensitivity class was then determined for each population. Each sensitivity class represents the percentage conidia resistant to the lower dose used, but sensitive to the upper dose (Steva, 1994). Previous studies (Shabi & Gaunt, 1992; Steva, 1994; Gubler *et al.*, 1996; Erickson & Wilcox, 1997) showed that the median 50% effective dose (ED_{50}) values for *U. necator* populations sensitive to DMIs is ≤ 0.3 µg/mL. A discriminating dose of 0.3 µg/mL was therefore used to designate variants as sensitive or resistant to a given fungicide. Variants catagorised in the 0–0.1 and 0.1–0.3 µg/mL range were considered as sensitive, whereas those catagorised in the 0.3–1.0 µg/mL and higher dose ranges were considered as resistant.

Statistical analysis: Correspondence analysis (Greenacre, 1985) was used to group populations of *U. necator* showing similar levels of reaction to the fungicides. The data were the number of spores reacting to the given dose and were entered as a matrix with the discriminatory doses as the columns and the regions as the rows for each fungicide. Probit analysis (Finney, 1972) was used with the aid of the POLO-PC computer program (LeOra Software, Berkeley, California) to estimate ED_{50}, ED_{90} and relative potency values of the fungicides to the different populations. Initially the full data set for each of the three fungicides was

analysed. If the probit lines were not parallel, the nine populations were arranged in order of increasing slopes. Data for the first two populations were then analysed. If the lines were parallel, data for the remaining populations were successively added until the assumption of parallelism was rejected. The data for the set of populations with parallel probit regression lines were then analysed to estimate the relative potencies. This was repeated for the data pertaining to the remaining set of populations. In this way the data for the nine populations were analysed in groups of parallel probit lines. Relative potencies of the fungicides to the different populations could only be estimated within groups of populations with parallel probit lines (Finney, 1972). The response of populations to the fungicides which did not have parallel probit lines was compared using the ED_{50} and ED_{90} values with their respective fiducal limits.

RESULTS

Survey of fungicides and vineyards in viticultural regions: A range of DMIs was used in 1996 by producers in the viticultural regions for the control of powdery mildew (Table 1). Triazole fungicides accounted for 86%, pyridines for 9%, and pyrimidines for 5% of the tonnage sold. Penconazole was the fungicide most commonly applied and accounted for 60% of the tonnage. DMIs were used more extensively by the table-grape than by the wine-grape industry. Five applications at a 21-day spray interval, or 7 applications at a 14-day spray interval were recommended and used by most producers. Most producers abandoned the use of sulfur in 1978 with the registration of triadimefon, which was used extensively in all the viticultural regions until 1983, when poor control was reported (M. Gordon, Bayer, *personal communication).* Registration of propiconazole in 1981, penconazole in 1983, hexaconazole in 1986, myclobutanil in 1987 and flusilazole in 1988 followed, which led to the minimal usage of triadimefon and triadimenol. The latter fungicide was applied for at least 10 years not as the only fungicide, but as a mixture in combination with tebuconazole. A few vineyards were identified in the Worcester-Robertson region as good candidates for obtaining isolates with baseline sensitivity due to the non-usage of DMIs. However, preliminary tests conducted with triadimenol as a tester fungicide on *U. necator* collected from these vineyards indicated slight shifts in sensitivity to this fungicide. Based on this information a small vineyard located 30 km from the commercial viticultural regions and isolated by two mountain ranges was selected in the Ceres Karoo region. Triadimefon was occasionally used in the vineyard prior to 1989, then abandoned. No other DMI was applied. The vineyards used for the collection of *U. necator* populations with suspected practical resistance to DMIs were all exposed to triadimefon or triadimenol prior to approximately 1989, when the use of these fungicides was terminated. They were then treated with different DMIs (Table 2), of which penconazole was the most widely used.

Fungicide sensitivity:

Triadimenol: The Ceres Karoo population, which was previously unexposed to DMIs other than triadimefon, consisted of four variant groups (Fig. 1): 18% were sensitive and belonged to the 0–0.1 and 0.1–0.3 µg/mL range; 50% belonged to the 0.3–1.0; 20% to the 1.0–3.0; and 12% to the 3.0–10.0 µg/mL resistance classes. In the Tulbagh and Paarl P populations, which were exposed annually to an average of three DMI applications, variants occurred in nearly all the resistance classes, but with the majority belonging to the middle range (0–0.1 to 30.0–50.0 µg/mL). In the populations exposed to an average of seven DMI applications (Paarl K, Paarl I, De Doorns, Riebeeck Kasteel), few conidia were grouped in any of the sensitive classes. Variants belonged primarily to the 3.0–10 to 50.0–100 µg/mL resistance classes. The Paarl I and Paarl K populations were not tested for

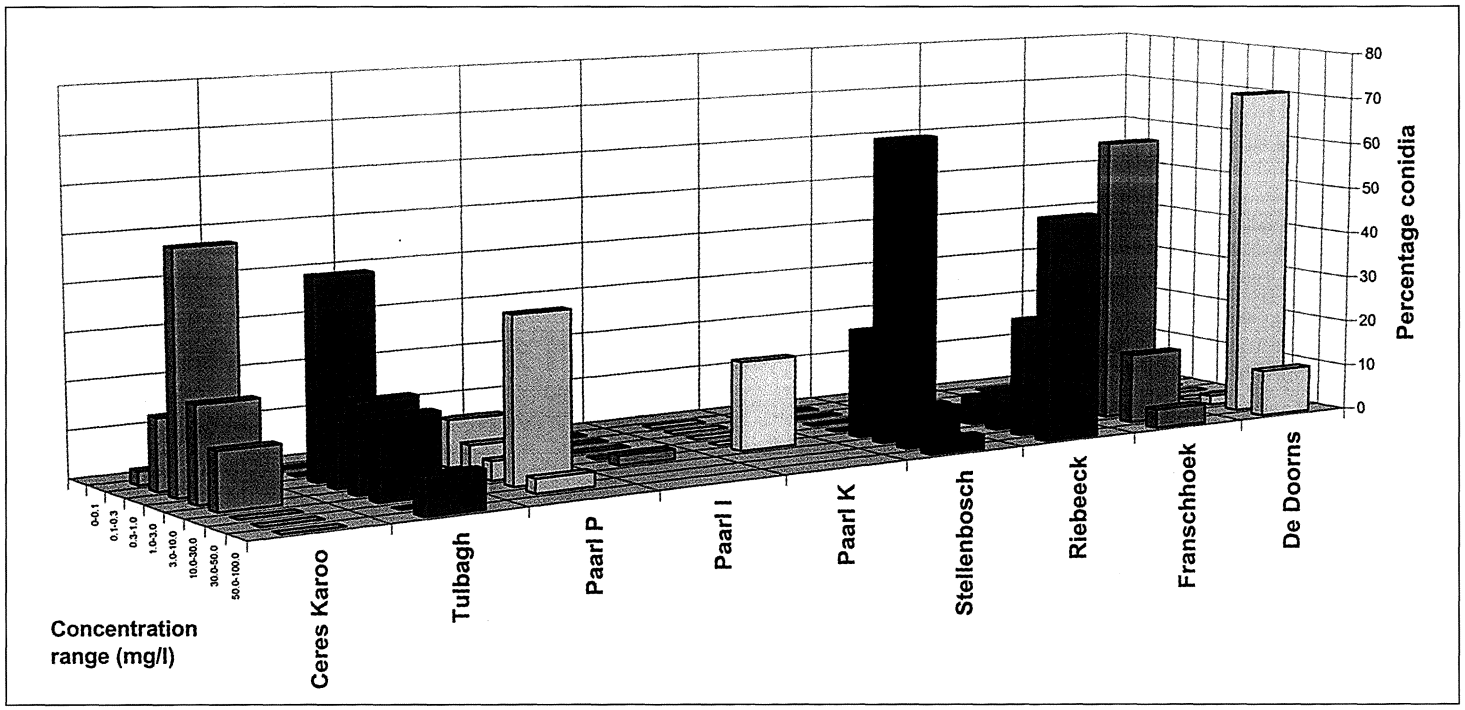

FIGURE 1

Percentage conidia belonging to different triadimenol sensitivity classes. Each bar represents the percentage conidia resistant to the lower dose, but sensitive to the upper dose.

sensitivity at the 50 and 100 µg/mL discriminatory doses. However, only a fraction of variants occurred in the ≤3.0–10.0 µg/mL range, which indicated a shift toward the resistant end of the spectrum. The De Doorns population showed the highest shift of distribution toward the resistant end of the spectrum, with 71% of the variants belonging to the 30.0–50.0, and 10% to the 50.0–100 µg/mL resistance classes.

The unexpectedly high variability in the response of the selected *U. necator* populations to triadimenol resulted in the use of different ranges of concentrations of this chemical in the experiment. The result was that a correspondance analysis of the data could not be performed.

Penconazole: The Ceres Karoo population was highly sensitive to the fungicide, with 90% and 8% of the conidia grouped into the 0–0.1 and 0.1–0.3 µg/mL classes respectively (Fig. 2). In the populations regularly exposed to DMIs, more resistant variants were found. In the Paarl P population, which displayed moderate resistance to triadimenol and which received only three DMI sprays early in the season, 60% of the population was still highly sensitive to penconazole. In the Stellenbosch population, which was highly resistant to triadimenol and unexposed to penconazole, nearly 82% of the population was in the 0.3–1.0 and 1.0–3.0 µg/mL resistance classes. The De Doorns population, which showed the highest shift in sensitivity to triadimenol, also showed the highest shift in sensitivity to penconazole.

A high percentage of the inertia (85.24%) was represented in the first dimension. Therefore this was the only axis plotted (Fig. 3). The Ceres Karoo and Paarl P populations were grouped together on the left of the centroid, while the other populations were on the right of the centroid. This suggests that the Ceres Karoo and Paarl P populations were of similar sensitivity and differed in sensitivity from the other populations. As the Ceres Karoo population was at baseline sensitivity, the Paarl P population, which only received two triazole sprays, was also assumed to be sensitive. These conclusions were supported by the probit analysis as 95% fiducal limits for the ED_{50} values of the Ceres Karoo and Paarl P populations

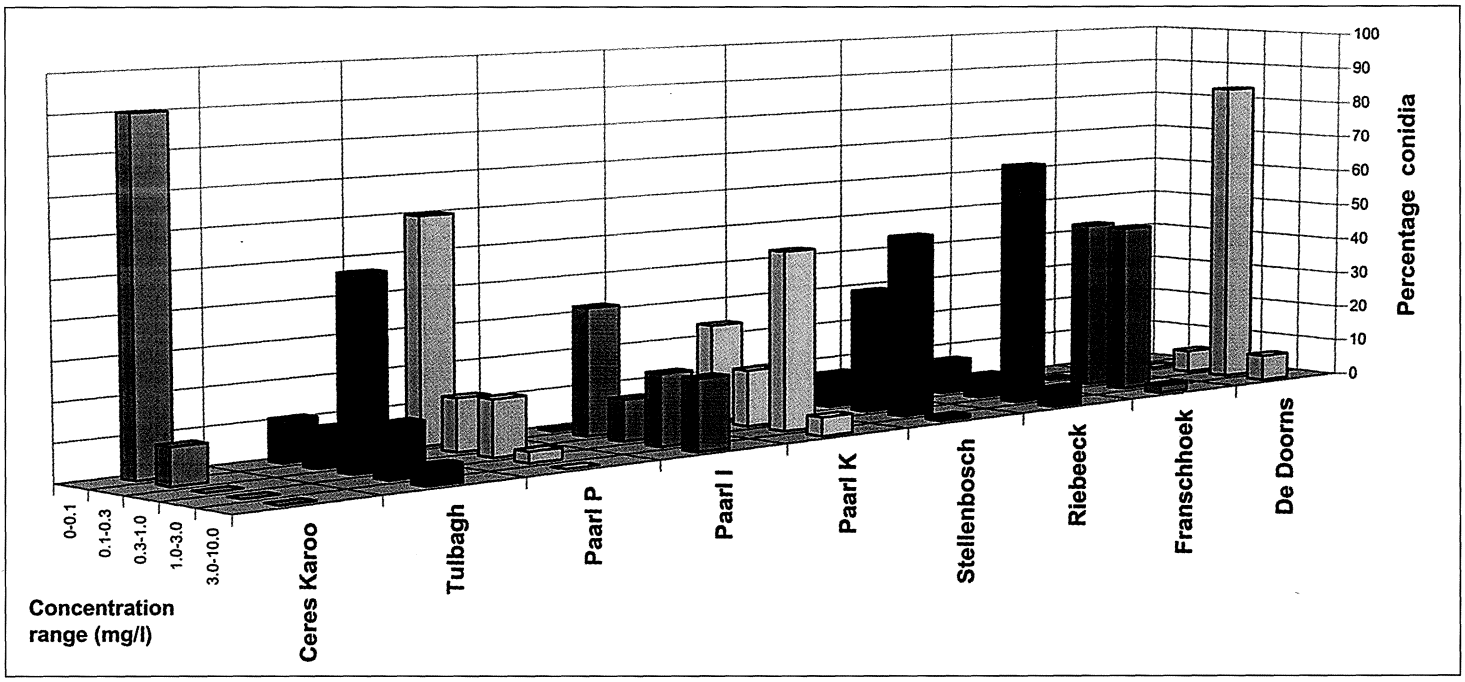

FIGURE 2

Percentage conidia belonging to different penconazole sensitivity classes. Each bar reresents the percentage conidia resistant to the lower dose, but sensitive to the upper dose.

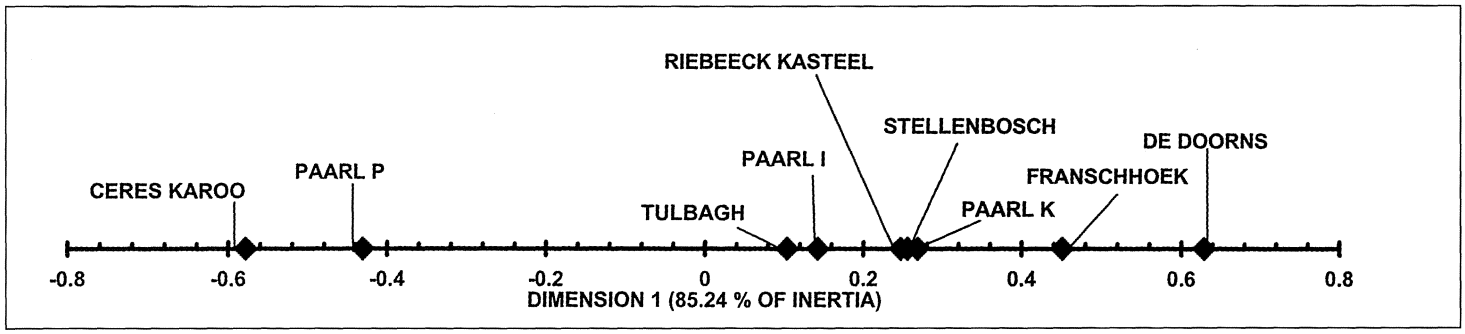

FIGURE 3

Correspondence in the reaction to penconazole of nine populations of *Uncinula necator* from different regions.

TABLE 3

Probit regression equations, ED_{50} and ED_{90} values (µg/mL) with their 95% fiducal limits obtained in the maximum likelikhood regression of probit mortality on log concentration of penconazole using nine poplations of *Uncinula necator.*

Locality	Probit regression	ED_{50}	95% Fiducal limits	ED_{90}	95% Fiducal limits
Paarl I	4.055 + 0.963 (X)	1.022	0.345 – 2.096	21.878	7.211 – 742.167
Paarl P	6.914 + 1.672 (X)	0.072	0.027 – 0.149	0.428	0.203 – 0.981
Franschhoek	5.017 + 1.672 (X)	0.863	0.368 – 1.694	5.043	2.569 – 11.804
Paarl K	5.070 + 1.672 (X)	0.908	0.486 – 1.601	5.303	2.859 – 13.212
Riebeeck Kasteel	4.836 + 1.672 (X)	1.253	0.706 – 2.435	7.320	3.536 – 29.651
Tulbagh	5.577 + 2.141 (X)	0.537	0.328 – 0.837	2.134	1.340 – 3.948
Stellenbosch	5.271 + 2.141 (X)	0.747	0.507 – 1.106	2.964	1.885 – 5.744
De Doorns	3.784 + 4.222 (X)	1.942	1.0 – 3.481	3.910	2.44 – 20.189
Ceres Karoo	10.527 + 4.222 (X)	0.049	0.0007 – 0.099	0.098	0.036 – 0.256

overlapped, but did not overlap with those of the other populations (Table 3). The 95% fiducal limits for the Tulbagh, Stellenbosch, Franschhoek, Paarl K, Paarl I and Riebeeck Kasteel populations all overlapped, indicating that their ED_{50} values were not significantly different. These populations were also roughly grouped together in the correspondence analysis (Fig. 3). However, the ED_{50} values of the latter populations were higher than those for the Ceres Karoo and Paarl P populations, indicating that they shifted more towards the resistant end of the spectrum than the other two populations. Furthermore, the ED_{50} value of the De Doorns population was significantly higher than that of the Tulbagh population. The De Doorns population was also the extreme right of the centroid (Fig. 3) in the correspondence analysis. Therefore, it appears as if the correspondence analysis groups the populations from the most sensitive on the left to the most resistant on the right of the axis.

The slope of the probit regression line for the Paarl I population was lower than for all the other populations, which confirmed the genetic heterogeneity of the population relative to its response to the chemical (Table 3). The populations from Paarl P, Paarl K, Franschhoek and Riebeeck Kasteel had probit regression lines with a common slope. The 95% fiducal limits of the relative potencies for Paarl K, Franschhoek and Riebeeck Kasteel relative to Paarl P did not include 1, indicating that the former three populations were more resistant to penconazole at the 95% confidence interval than the Paarl P population. The populations from Tulbagh and Stellenbosch had probit regression lines with a common slope. The slope of the probit regression line for the De Doorns population was the highest, suggesting genetic homogeneity of the population relative to its reaction to penconazole. Therefore, because this population is highly resistant, the existence of a high proportion of resistance variants can be assumed.

The slope of the probit regression lines for the Ceres Karoo and the De Doorns populations were the highest, suggesting genetic homogeneity within these two populations relative to their reaction to penconazole. However, the estimate of relative potency (Table 4) indicates that the concentration of penconazole producing a given mortality of the Ceres Karoo population would have to be increased by 39.57 times to produce the same mortality in the De Doorns population.

TABLE 4

Relative potency of penconazole to pairs of populations of *Uncinula necator* with the 95% fiducal limits.

Populations[a]	Relative potency	95% Fiducal limits
Franschhoek relative to Paarl P	12.051	4.413 – 34.770
Paarl K relative to Paarl P	12.673	5.078 – 37.299
Riebeeck Kasteel relative to Paarl P	17.489	6.831 – 61.387
De Doorns relative to Ceres Karoo	39.57	20.146 – 316.348[b]

[a] The first population of each pair is the most resistant.

[b] 90% Fiducal limits.

Flusilazole: The Ceres Karoo population consisted of three variant groups (Fig. 4). The main group, which consisted of 84% of the population, was still highly sensitive and belonged to the 0.1–0.3 and the 0.3–1.0 µg/mL sensitivity classes. The other group consisted of resistant variants belonging to the 0.3–1.0 µg/mL (16%) resistance class. Populations which displayed substantial shifts in reduced sensitivity to triadimenol, and that were unexposed to flusilazole, displayed substantial shifts in reduced sensitivity to flusilazole. In the Tulbagh, Paarl P, Paarl I and Paarl K populations, approximately 30–40% of the resistant variants belonged to the two highest resistant classes (1.0–3.0 and 3.0–10.0 µg/mL). In the Franschhoek population, approximately 70% of the population consisted of resistant variants belonging to the latter resistant classes. In the Riebeeck Kasteel and De Doorns populations, which were also highly resistant to triadimenol, but which were exposed to flusilazole as well as other DMIs, ≥ 80% of the population belonged to the 1.0–3.0 and 3.0–10.0 µg/mL resistant classes.

A high percentage of the inertia (86.51%) was represented in the first dimension. Therefore, this was the only axis plotted (Fig. 5). The Ceres Karoo population was to the extreme left of the axis and the Franschhoek population at the extreme right. The ED_{50}

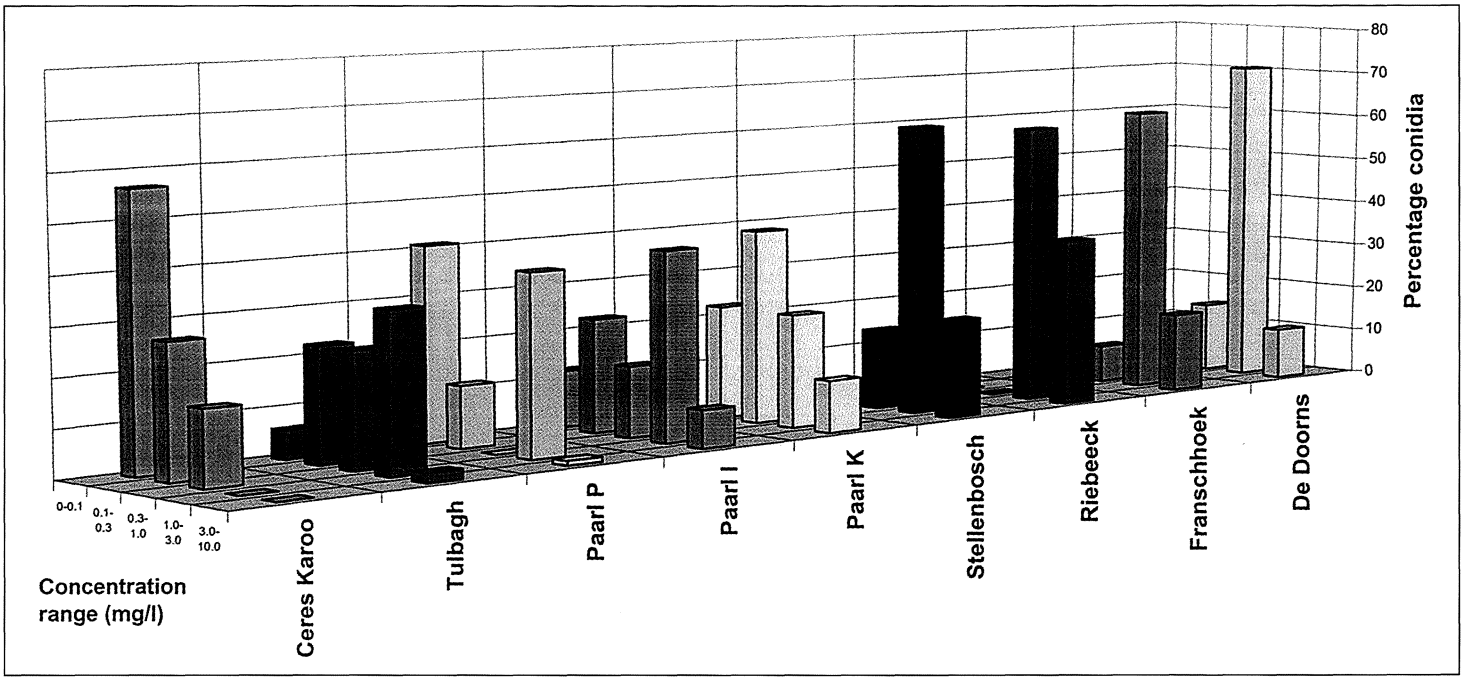

FIGURE 4

Percentage conidia belonging to different flusilazole sensitivity classes. Each bar represents the percentage conidia resistant to the lower dose, but sensitive to the upper dose.

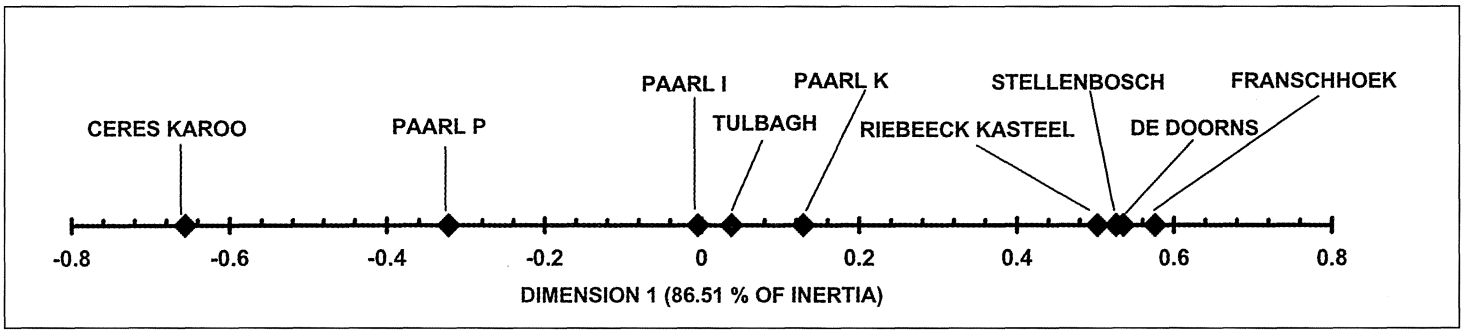

FIGURE 5

Correspondence in the reaction to flusilazole of nine populations of *Uncinula necator* from different regions.

value for the Ceres Karoo population was lower than that for the others, suggesting that the Ceres Karoo population was the most sensitive. The 95% fiducal limits could not be estimated for the Paarl P and Paarl I populations due to excessively high heterogeneity factors (Finney, 1972). The 95% fiducal limits for the ED_{50} values of the Ceres Karoo population did not overlap with those of the remaining populations, indicating that the Ceres Karoo population was significantly more sensitive than the remaining populations. The 95% fiducal limits of the ED_{50} values for the Franschhoek population did not overlap with those for the Tulbagh or Paarl K populations (Table 5) indicating that the latter two were significantly more sensitive than the Franschhoek population. The 95% fiducal limits of the ED_{50} values for the Riebeeck Kasteel, Stellenbosch, De Doorns and Franschhoek populations overlapped indicating that differences in the ED_{50} values were not significant, and these four populations were grouped together in a cluster by the correspondence analysis. The population with the highest ED_{50} value was from Franschhoek, indicating that it was the most resistant population. This also supports the conclusion drawn from the correspondence analysis. Therefore, it appears as if the correspondence analysis positions the populations from left to right along the axis with the most sensitive to the left and the most resistant to the right.

The slopes of the probit regression lines for the Paarl P and Paarl I populations were lower than those of all the other populations suggesting a high degree of heterogeneity with respect to sensitivity to flusilazole. The high heterogeneity factors (34.87 for Paarl P and 47.76 for Paarl I) (Finney, 1972) estimated in the probit analysis confirms the conclusion and also supports the inferences made from Fig. 4. The slope of the regression line for the Stellenbosch population was the highest. This, together with the high ED_{50} value (Table 5), suggests a high degree of homogeneity with respect to the presence of resistance alleles in the Stellenbosch population.

TABLE 5

Probit regression equations, ED_{50} and ED_{90} values (µg/mL) with their 95% fiducal limits obtained in the maximum likelikhood regression of probit mortality on log concentration of flusilazole using nine poplations of *Uncinula necator.*

Locality	Probit regression	ED_{50}	95% Fiducal limits	ED_{90}	95% Fiducal limits
Paarl I	5.336 + 0.895 (X)	0.917	–	24.773	–
Paarl P	5.779 + 1.158 (X)	0.212	–	2.718	–
Tulbagh	5.424 + 1.917 (X)	0.601	0.444 – 0.792	2.801	2.011 – 4.342
Ceres Karoo	7.101 + 1.917 (X)	0.080	0.054 – 0.111	0.374	0.273 – 0.543
Paarl K	5.331 + 2.347 (X)	0.722	0.311 – 1.647	2.540	1.170 – 9.653
Riebeeck Kasteel	4.223 + 2.347 (X)	2.143	0.938 – 5.283	7.535	3.325 – 32.871
Franschhoek	3.617 + 2.347 (X)	3.885	1.674 – 9.819	13.661	5.927 – 61.166
De Doorns	4.099 + 3.487 (X)	1.813	0.871 – 3.568	4.227	2.458 – 32.663
Stellenbosch	4.041 + 4.830 (X)	1.580	1.485 – 1.680	2.910	2.673 – 3.217

TABLE 6

Relative potency of flusilazole to pairs of populations of *Uncinula necator* with the 95% fiducal limits.

Populations[a]	Relative potency	95% Fiducal limits
Tulbagh relative to Ceres Karoo	7.499	4.968 – 11.863
Riebeeck Kasteel relative to Paarl K	2.966	0.097 – 1.070
Franschhoek relative to Paarl K	5.378	1.677 – 19.131

[a] The first population of each pair is the most resistant.

An estimate of the effectivity of flusilazole in a population (Tulbagh) exhibiting a slight shift relative to that of a population at baseline sensitivity (Ceres Karoo), indicates that the concentration of the fungicide required to produce a given mortality for the Ceres Karoo population would have to be increased by 7.499 times to produce the same mortality in the Tulbagh population (Table 6).

DISCUSSION

The approach of assaying large sample sizes of *U. necator* populations in commercial vineyards with a history of poor disease control for the distribution of variants resistant to the most commonly used DMIs, and to calculate the concentration of fungicide required to produce a given mortality for these populations relative to a sensitive one, provided a perspective on the DMI resistance phenomenon in local viticultural regions. Resistance of *U. necator* to DMI fungicides has not been studied previously in South Africa, in spite of reports from producers during 1983–1985 of poor powdery mildew control by triadimefon in the De Doorns region of the Hex River valley (M. Gordon, Bayer, *personal communication).* The finding that variants resistant to triadimenol occurred at high frequencies after nearly 10 years of non-usage in a vineyard in this region indicates an earlier shift in triadimenol sensitivity in the regional subpopulation. Furthermore, similar shifts displayed to triadimenol by subpopulations from other viticultural regions may point to the earlier introduction and establishment of resistant variants in different geographical regions. The resistant variants also appeared to be sufficiently competitive to become established in vineyards of the sub-regions. In the Western Cape province, grapevine is cultivated predominantly in five regions: the Hex River valley, Tulbagh-Worcester-Robertson valley, Paarl-Franschhoek valley, Stellenbosch region and Riebeeck Kasteel region. The Hex River region is separated from the Tulbagh-Worcester-Robertson region by mountain ranges. The same holds for the Paarl-Franschhoek, Stellenbosch and Riebeeck Kasteel regions, which are separated from the former group. Cultivation in each of these geographical regions is extensive, therefore wind may play a prominent role in the dispersal of conidia of resistant variants within each of the regions. This is substantiated by preliminary data (F. Halleen, unpublished) that *U. necator* in vineyards offered by producers as good candidates for obtaining isolates at baseline sensitivity due to the non-usage of DMIs, or where only sulphur was applied, displayed slight shifts in triadimenol sensitivity. Wind dispersal of conidia over long distances has not been demonstrated for *U. necator,* but has been reported for *Erysiphe graminis* f. sp. *hordei* in northern Europe (Wolfe & McDermott, 1994).

Although single-conidial isolates were not examined for cross-resistance in this study, cross-resistance between triadimenol, penconazole and flusilazole is indicated by the frequency at which variants resistant to the respective fungicides were found in the subpopulations. The Ceres Karoo population, which displayed a slight shift in sensitivity to triadimenol but had not received DMIs for the last 10 years, was at baseline sensitivity level for penconazole and flusilazole. On the other hand, the four populations (De Doorns, Franschhoek, Riebeeck Kasteel and Stellenbosch) which showed the highest shifts towards the resistant end of the spectrum for triadimenol, also displayed a high level of reduced sensitivity to flusilazole. This was in spite of the fact that only the Stellenbosch population was regularly treated with flusilazole. The other three populations were primarily exposed to penconazole. Reduced sensitivity to penconazole was furthermore most prevalent in the Paarl K, Paarl I, Riebeeck Kasteel and De Doorns populations. Of these populations, Paarl K and Paarl I received primarily penconazole, whereas the other two populations were treated with a range of DMIs. The data also indicated differing

sensitivity of the pathogen to the fungicides used. ED_{50} values for penconazole for the Paarl K, Paarl I, Riebeeck Kasteel and De Doorns pathogen populations, which showed the highest shifts in sensitivity to this fungicide, were 0.908, 1.022, 1.253 and 1.942 µg/mL, respectively. In these populations, 53%, 38%, 71% and 91% respectively of the conidia belonged to the 1.0–3.0 µg/mL and higher resistant classes. Reduced sensitivity to flusilazole was most prevalent in the Stellenbosch, De Doorns, Riebeeck Kasteel and Franschhoek populations. ED_{50} values for these populations were 1.580, 1.813, 2.143, 3.885 µg/mL, respectively. For flusilazole, on the other hand, 83%, 82%, 96% and 79% respectively of the conidia belonged to the 1.0–3.0 µg/mL and higher resistant classes. These differences in sensitivity occurred in spite of the fact that most of these populations were subjected to the almost exclusive use of penconazole (7 to 9 consecutive sprays per season) since its introduction in 1983, whereas triadimefon had not been used since 1989. These findings point to a differing sensitivity of the pathogen to triadimenol, penconazole and flusilazole (triazoles), and confirmed previous reports (Erickson & Wilcox, 1997; Ypema *et al.,* 1997) which indicate that resistance to DMIs is a multigenic trait in *U. necator* and that one or more resistance genes are independent with respect to individual DMIs or groupings of them. This has significant implications with respect to programmes for the management of DMI resistance (Erickson & Wilcox, 1997).

Cleistothecia of *U. necator* were first observed in South Africa in the Stellenbosch region during 1996 (Halleen & Holz, 2000). Their sparse and sporadical formation very late in the growing season was ascribed (Halleen & Holz, 2000) to the fact that an opposing mating type might have been introduced only recently, and that opposing mate types are not yet abundant in local vineyards. The common occurrence of triadimenol-resistant variants in problem vineyards selected from the main grapevine-growing regions suggests that cleistothecia may be more widely formed than originally reported (Halleen & Holz, 2000) and that they may contribute to the perpetuation of triadimenol resistance in the local *U. necator* population. Gubler and Ypema (1996) demonstrated that increased triadimefon resistance is perpetuated through the sexual cycle of the pathogen, and suggested that DMI resistance can be maintained in overwintering ascospores. Therefore, apart from wind dispersal, resistant variants can also be dispersed over long distances in the form of cleistothecia on the bark of plant material, or dormant mycelium in grape buds (Bulit & Lafon, 1978). The fact that variants resistant to triadimenol, penconazole and flusilazole were detected in each region showed that the emphasis should be placed in the viticultural regions on the control of *U. necator* on imported plant material.

Poor control of *U. necator* with DMI fungicides has been associated with sensitivity shifts and the possibility of practical resistance in vineyards in the USA (Erickson & Wilcox, 1997; Ypema *et al.,* 1997), France (Steva, 1994) and Austria (Redl & Steinkellner, 1996). The practical resistance phenomenon occurs once the frequency of resistance phenotypes precludes commercially acceptable disease control under standard usage regimes (Brent, 1995). In neither of these vineyards has the relationship been quantified between a vineyard population's sensitivity to a DMI fungicide and the fungicide's ability to control the pathogen in the vineyard. Estimates of the effectivity of penconazole and flusilazole on resistant populations relative to the Ceres Karoo population, which was at baseline sensitivity to the two fungicides, clearly indicate practical resistance in most of the vineyards used in this study. For example, the concentration of penconazole required to produce a given mortality of the De Doorns population (ED_{50} value of 1.942 µg/mL) would have to be increased by 39.57 times to produce the same mortality as in the Ceres Karoo population (ED_{50} value of 0.049 µg/mL). Thus, the practical significance of resistance to DMI fungicides can be determined conclusively only by monitoring shifts in sensitivity distributions and fungicide performances in relevant field experiments in the different viticultural regions.

Most producers appeared to be adhering to the recommendations of Nietvoorbij (De Klerk, 1988) which advocate 5–7 applications per season. This study showed that by following these guidelines, resistance developed in *U. necator* to triadimenol, penconazole and flusilazole in South African grapevines. Thus, to ensure that DMIs can remain as an effective basis for the control of powdery mildew, a maximum of three applications per season is suggested as recommended by Clerjeau (1994). The introduction of the new strobilurin fungicides, azoxystrobin and kresoxim-methyl (Ypema, 1999) and the biological agent *Ampelomyces quisqualis* formulated as AQ10™ (Daoust & Hofstein, 1996) represent important new tools available for the control of grapevine powdery mildew. Recent studies also indicate that substantial ontogenic resistance is expressed much earlier than previously believed, which narrows the period of susceptibility (Gadoury, 1998). A better understanding of the epidemiology of the fungus, together with the availability of a wider range of products with different modes of action, will eventually lead to better powdery mildew control and management of fungicide resistance.

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