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Observations on the behaviour of different populations of *Plasmopara viticola* resistant to QoI fungicides in Italian vineyards

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Summary. Grapevine downy mildew, caused by *Plasmopara viticola,* is probably the most damaging fungal disease of grapevine world-wide. Among the fungicides recently developed for downy mildew control is the QoI class of fungicides, which inhibits mitochondrial respiration. Since 1999, selected *P. viticola* populations in northern Italy have been monitored for resistance to QoI fungicides. Detached leaf discs and whole potted plants were used under controlled conditions to test the sampled populations. QoI-resistant populations of *P. viticola* were found in all the vineyards sampled in 2001 and 2002 in Trentino Alto Adige and Friuli Venezia Giulia, where failure in QoI control was reported. Many of the populations had minimum inhibition concentration (MIC) values 3– 30 times higher than those of sensitive reference populations. Populations of *P. viticola* sampled from vineyards in Piedmont, where no QoI fungicides had previously been used, showed MIC values equal to, or lower than those of the reference populations. Most of the *P. viticola* populations collected in Trentino Alto Adige in 2001 showed high virulence in leaf disc test and were not controlled by QoI fungicides, applied both at field and double field rates in the whole plant test. Most of these populations retained their virulence in the subsequent leaf disc test in water.

Key words: grapevine, downy mildew, strobilurines, resistance.

Introduction

Grapevine downy mildew, caused by *Plasmopara viticola* (Berk. & Curtis *ex.* de Bary) Berl. & de Toni, is probably the most damaging fungal disease of grapevine world-wide (Hewitt and Pearson, 1988). In Italy, the disease is particularly severe in the Northern regions causing almost complete loss of yield in some areas and in the absence of treatment, as observed in Piedmont in 1994 and 1998 (Monchiero *et al.,* 1999). Damage is particularly severe when warm and wet conditions occur in late spring and summer.

Chemical control is necessary, but the choice of fungicide must be carefully considered: copper-based fungicides, for instance, are slightly phytotoxic to some cultivars and, since copper is not degradable, can lead to copper accumulation in vineyard soils. Folpet and the ethylenebisdithiocarbamates are under increasing regulatory scrutiny. Resistance to phenylamide fungicides such as metalaxyl has been detected since the early 1980s (Leroux and Clerjeau, 1985; Gisi, 2002). In Italy, resistance towards this group of fungicides was observed in northern Italy (Mezzalama *et al.,* 1991), without however causing severe problems. In 1993, disease-control failures were observed when cymoxanil was applied in postinfection sprays in mixture with copper or mancozeb (Gullino *et al.,* 1997). Thus

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it is obvious that action is needed to maintain the current efficacy of the fungicides used against downy mildew. Among the fungicides recently developed for downy mildew control is the QoI class of fungicides, which bind to the Qo site of the cytochrome bc_1 complex and inhibit mitochondrial respiration by blocking electron flow through the electron transport chain (Becker *et al.,* 1981; Von Jagow *et al.,* 1986; Brandt *et al.,* 1988; Heaney *et al.,* 2000; Gisi, 2002). In Italy, the QoI fungicides most commonly applied against grape downy mildew are azoxystrobin, trifloxystrobin, famoxadone and fenamidone. Azoxystrobin was the first QoI fungicide to be registered for use in Italy (Gullino and Garibaldi, 2003). The QoI fungicides, due to their highly specific mode of action, are considered at risk of resistance developing against them (Gisi, 2002). Field resistance has already developed in *Erysiphe graminis* (Chin *et al*., 2001a; Sierotsky *et al*., 2000), *Plasmopara viticola* (Gullino *et al*., 2001; Heaney *et al*., 2000), *Pseudoperonospora cubensis* (Ishii *et al*., 2001), *Mycosphaerella fijiensis* (Chin *et al*., 2001b) and *Venturia inaequalis* (Kung Farber *et al*., 2002). Cross-resistance to all QoI inhibitor fungicides has been observed in many pathogens (Gisi, 2002). In all these pathogens, a single amino-acid exchange, G143A, seems to induce QoI resistance (Sierotzki *et al*., 2000; Gisi *et al*., 2002; Heaney *et al*., 2000). However, other resistance mechanisms and additional mutations are likely to occur in some other plant pathogens (Gisi, 2002). For instance, an additional amino-acid change from phenylalanine to leucine at position 129 was detected conferring resistance to QoI in *Pyricularia grisea* (Farman, 2001).

Since 1999, in three regions of northern Italy, the sensitivity of various populations of *P. viticola* has been monitored, first to determine their baseline sensitivity to QoI compounds, and then to ascertain whether any changes were occurring in the efficacy of these compounds against downy mildew. In 2001, resistance was detected in commercial vineyards of Trentino Alto Adige and Friuli Venezia Giulia (Gullino *et al.,* 2001).

Resistance to QoI must now be prevented and managed in order to permit a longer effectiveness of QoI fungicides. Monitoring fungal pathogen populations for changes of sensitivity to QoI is part of integrated pest management (IPM) strategies.

Therefore, it seemed interesting to investigate the resistance of *P. viticola* to QoI fungicides with the objective of evaluating: 1) the extent of the resistance problem in commercial vineyards; 2) the *in vitro* sensitivity of *P. viticola* to azoxystrobin and other QoI fungicides, in order to detect the cross-resistance patterns; 3) sporulation of populations of *P. viticola* after transfer to the leaves; 5) the sensitivity of *P. viticola* to three QoI fungicides (azoxystrobin, trifloxystrobin and famoxadone) on grape plants.

Materials and methods

Sampling

In 1999–2002, leaves with early symptoms of downy mildew and bearing freshly sporulating lesions were collected randomly in several commercial vineyards from three regions in northern Italy: Piedmont, Trentino Alto Adige and Friuli Venezia Giulia. The samples collected consisted of a mixture of fungal genotypes. Twenty to fifty leaves of the same age were taken from about different vines in each vineyard.

In Trentino Alto Adige 21 vineyards were sampled in 1999 (Table 1), 24 in 2001 and 9 in 2002 (Table 2). In Friuli Venezia Giulia 4 vineyards were sampled in 2001 (Table 3). In Piedmont 13 vineyards were sampled in 2000 and 12 in 2002 (Table 4).

Eight of the vineyards sampled in Trentino Alto Adige in 2001 were sampled again in 2002.

In Trentino Alto Adige and Friuli Venezia Giulia, those vineyards were sampled where control failures of QoI compounds had been observed or reported.

Mildew incidence in the field was determined as the percentage of infected bunches in a total of 50 bunches per vineyard, by technicians from the extension service of each region.

After sampling, sporangia from infected leaves were brushed into sterile distilled water with a sterile brush and inoculated on test leaf discs or plants within 24 h. Populations of *P. viticola* were maintained in a suspension of milk and glycerol (10 ml glycerol, 8.5 g of skim-milk and 90 ml distilled water) at -18°C.

One population of *P. viticola* was sampled every year from grapevines cv. Moscato grown in a family orchard in Piedmont never treated with QoI fungicides, and used as the reference (sensitive) control.

Table 1. Leaf disc test. Minimum inhibitory concentration (MIC) values, expressed in mg l^{-1} of azoxystrobin, in 21 populations of *Plasmopara viticola* collected from vineyards in Trentino Alto Adige in 1999.

Population	Cultivar	MIC (mg l^{-1})
Reference 1999[a]	Moscato giallo	1.3
1	Chardonnay	5.3
2	Merlot	1.3
3	Schiava	5.3
4	Chardonnay	1.3
5	Muller Thurgau	1.3
6	Moscato giallo	5.3
3	Schiava	5.3
7	Teroldego	5.3
8	Traminer	1.3
9	Merlot	21.3
6	Enantio	2.7
10	Chardonnay	1.3
11	Pinot grigio	<1.3
12	Pinot bianco	10.7
13	Merlot	1.3
14	Merlot	1.3
15	Pinot nero	5.3
16	Chardonnay	21.3
17	Chardonnay	5.3
18	Cabernet	1.3
19	Cabernet	1.3

[a] Sensitive population of *P. viticola* sampled every year from grapevines cv. Moscato grown in a family orchard in Piedmont never treated with QoI fungicides, and used as a reference.

Leaf disc test

The leaf disc test was used for monitoring in all 3 years. Suspensions of sporangia were brushed into distilled water from at least 20 randomly collected infected leaves bulked together. Leaf discs (1.5 cm diameter) were cut with a cork borer from healthy, fully expanded leaves (15–20 cm wide) of potted grapevine plants cv. Chardonnay that had not been treated with downy mildew fungicides. These plants were treated with sulphur three or four times per season to prevent powdery mildew. Leaf discs were dipped into a distilled water suspension of the fungicide for twenty minutes.

In vitro *evaluation of the sensitivity of* P. viticola *to QoI fungicides*.

In order to test baseline sensitivity to QoI fungicides, a commercial formulation of azoxystrobin (Quadris 250 SC) was used at 1/4, 1/16, 1/32 and 1/64 of the field rate, corresponding to 43.0, 10.6, 5.3 and 2.7 mg l^{-1} a.i. respectively. Concentrations of 0; 0.01; 0.03; 0.1; 0.3; 1; 3; 10; 30; 100; 300 and 1000 mg a.i. l^{-1} were used for further sensitivity testing. The control discs were dipped into deionised sterilised water.

When dry, each leaf disc was inoculated on the lower leaf surface with 3 droplets (10 μl each) of sporangial suspension (1.5×10^5 sporangia ml^{-1}). Ten leaf discs per concentration were inoculated and placed in 9-cm diameter plastic Petri dishes (five discs per dish), floating, upper surface down, on the same fungicide suspension as that used for treatment.

Discs were incubated in growth chambers at 23°C until symptom appearance. Disease severity was recorded after 7 and 10 days by calculating the percentage of disc surface showing *P. viticola* sporulation.

For each population, the minimum inhibitory concentration (MIC) [minimum fungicide concentration (mg l^{-1}) that completely inhibited the sporulation of *P. viticola* on leaf discs] of the tested fungicides was calculated. The MIC for each population was assessed only when all 10 untreated discs sporulated. All doubtful cases were sampled and tested again.

Evaluation of the sporulation capacity of populations of P. viticola

The populations of *P. viticola* collected in Trentino Alto Adige in 2001 and 2002 were kept at -18°C and then propagated on leaves or on healthy grape plants kept at 20°C and at high relative humidity (90% RH). The same populations were subsequently inoculated on leaf discs of healthy vines floating on water and kept in a growth chamber at a constant 20°C. At the onset of sporulation, which occurred at varying times after inoculation of the different populations, sporangia were collected in order to obtain a final suspension between 5×10^4 and 1×10^5 sporangia ml^{-1}.

After dilution of the starting suspension of sporangia, leaf discs floating on water were inoculated (10 discs per each concentration) with 1×10^3, 5×10^3, 1×10^4, 5×10^4 and 1×10^5 sporangia ml^{-1}. Data collected after 12 days were expressed as average per cent leaf area showing infection with downy mildew.

Table 2. Leaf disc test. Minimum inhibitory concentration (MIC) values, expressed as mg l^{-1} of azoxystrobin, in populations of *P. viticola* collected from vineyards in Trentino Alto Adige in 2001 and 2002.

Population	Cultivar	Infected bunches (%)[a]	Treatments with QoI fungicides			MIC (mg l^{-1})
			First year of use	No. of treatments per year	Total No. of treatments after first year of use	
Year 2001						
Reference 2001[b]	Moscato giallo	-	Not used	Not used	Not used	30
2	Chardonnay	10	1998	4	13	1000
3	Chardonnay	47	1998	2	7	>1000
4	Sortesele	59	Not used	Not used	Not used	>1000
5	Teroldego	100	1998	3	13	>1000
7	Chardonnay	7	1998	1	7	>1000
8	Pinot grigio	80	2001	4	4	300
10	Merlot	100	Not used	Not used	Not used	300
11	Chardonnay	<5	2001	5	5	>1000
12	Nosiola	<5	1999	2	6	300
13	Pinot grigio	<5	1998	3	12	>1000
16	Chardonnay	<5	1999	3	7	>1000
17	M. Thurgau	60	Not used	Not used	Not used	100
18	Teroldego	n.d.	2000	3	7	>1000
19	Teroldego	100	1998	3	13	1000
22	Pinot bianco	80	Unknown	4	Unknown	>1000
24	Teroldego	n.d.	1998	4	14	>1000
25	Merlot	n.d.	2000	6	6	>1000
26	Merlot	n.d.	1998	5	11	>1000
29	Traminer Aromatico	<5	Unknown	4	Unknown	1000
30	Chardonnay	52	Unknown	4	Unknown	1000
32	Misto rosso	<5	1999	5	Unknown	>1000
33	Cabernet	<5	2000	4	4	>1000
34	Pinot grigio	80	Unknown	4	Unknown	>1000
35	Pinot grigio	<5	Not used	Not used	Not used	>1000
Year 2002						
Reference 2002[b]	Moscato giallo	-	Not used	Not used	Not used	10
2	Chardonnay	0	1998	Not used	13	300
3	Chardonnay	< 5	1998	Not used	7	300
4	Sortesele	100	Not used	Not used	Not used	100
5	Teroldego	100	1998	Not used	13	>300
8	Pinot grigio	80	2001	Not used	4	>300
10 bis	Chardonnay	5	1998	Not used	Not used	> 300
17	Müller Thurgau	< 5	Not used	Not used	Not used	>300
22	Pinot bianco	<5	Unknown	Not used	Unknown	>300
26	Merlot	5	1998	Not used	11	>300

[a] Evaluated by extension service on 50 bunches.
[b] Sensitive population of *P. viticola* sampled every year from grapevines cv. Moscato grown in a family orchard in Piedmont never treated with QoI fungicides, and used as a reference.
n.d., not determined.

Table 3. Leaf disc test. Minimum inhibitory concentration (MIC) values, expressed in mg l^{-1} of azoxystrobin, in populations of *P. viticola* collected from vineyards in Friuli Venezia Giulia in 2001.

Population	Cultivar	Infected bunches (%)[a]	Treatments with QoI fungicides			MIC (mg l^{-1})
			First year of use	In 2001	Total No. of treatments after first year of use	
Reference 2001[a]	Moscato		Not used	Not used	0	30
1I	Merlot	90	1998	3	Unknown	1000
1II	Tocai	60	1998	8	17	>1000
2II	Sauvignon	5	1999	4	10	>1000
4II	Merlot	75	2000	5	10	>1000

[a] See Table 2.
[b] See Table 2.

Table 4. Leaf disc test. Minimum inhibitory concentration (MIC) values, expressed as mg l^{-1} of azoxystrobin, in populations of *P. viticola* collected in 2000 and 2002 from Piedmont vineyards where QoI fungicides were not previously used.

Population	Cultivar	Infected bunches (%)[a]	MIC (mg l^{-1})
Year 2000			
Reference 2000[b]	Moscato giallo	-	30
1	Dolcetto	5	3
2	Moscato	<5	3
3	Moscato	5	3
4	Cortese	10	3
5	Cortese	5	1
6	Dolcetto	10	1
8	Dolcetto	10	1
15	Barbera	10	1
16	Barbera	10	3
20	Cortese	20	30
21	Barbera	30	1
22	Cortese	10	3
23	Chardonnay	10	10
Year 2002			
Reference 2002[b]	Moscato giallo	-	10
1	Barbera	< 5	0.3
3	Chardonnay	< 5	0.3
5	Cortese	5	1
7	Sangiovese	5	0.3
8	Cortese	< 5	1
9	Barbera	10	1
10	Unknown	10	1
11	Cortese	15	1
12	Cortese	10	1
13	Moscato	20	1
14	Barbera	20	3
15	Brachetto	20	3

[a] See Table 2.
[b] See Table 2.

Table 5. Leaf disc test. Mean incidence of *P. viticola* as percent leaf area showing downy mildew infection 12 days after inoculation with populations of *P. viticola* from vineyards located in Piedmont (P) in 2002, Friuli Venezia Giulia (F) in 2002 and Trentino (T) in 2001.

Population	Origin	Mean incidence (%)	Mean incidence per each sporangia concentration (sporangia ml^{-1})				
			1×10^3	5×10^3	1×10^4	5×10^4	1×10^5
1 P	P	n.d.	0	1	0	2	14
3 P	P	n.d.	0	0	0	7	14
5 P	P	n.d.	0	0	0	0	1
7 P	P	n.d.	7	6	16	20	31
8 P	P	n.d.	6	16	34	34	44
10 P	P	n.d.	0	3	2	31	36
11 P	P	n.d.	0	15	15	21	29
1 II	F	0.16 a[a]	0	0	0	0	4
4 II	F	6.5 bcd	3	7	8	10	6
3 T	T	13.6 ghi	1	11	4	22	32
4 T	T	0 a	0	0	0	0	0
5 T	T	1.2 a	0	0	0	2	5
6 T	T	12.5 ghi	0	9	7	23	25
8 T	T	0.4 a	0	0	0	0	2
10 T	T	10.6 defg	9	6	3	23	14
16 T	T	15.1 hi	0	0	8	35	32
17 T	T	1.2 a	0	0	2	3	2
18 T	T	0.9 a	1	2	1	1	1
19 T	T	1.3 a	0	0	0	2	5
22 T	T	11.7 efgh	2	9	10	19	12
23 T	T	0.5 a	0	2	0	0	1
25 T	T	9.3 def	0	2	10	23	13
26 T	T	4.6 abc	0	6	3	9	5
29 T	T	0 a	0	0	0	0	0
32 T	T	0 a	0	0	0	0	0
33 T	T	1.3 a	0	0	0	1	2
34 T	T	9.4 def	0	0	0	16	31
35 T	T	15.8 i	0	0	0	47	33
36 T	T	2.3 ab	0	3	0	7	5
Reference[b]		7.6 cde	0	2	2	13	22
Mean incidence (%)		n.d.	0.6 a[a]	2.3 b	2.5 b	11.0 c	10.9 c

[a] Values followed by the same letter are not statistically different by Tuckey's test ($P<0.05$).
[b] See Table 2

Whole potted-plant test

The sensitivity of populations of *P. viticola* to QoI fungicides was tested on potted grape plants. Populations of *P. viticola* collected in 2001 from different vineyards in Trentino Alto Adige were inoculated by spraying the lower surface of every leaf of healthy vines cv Chardonnay (grown in growth chambers at 20°C and 90% RH in 3 l pots) with sporangial suspension in distilled water (at least 1×10^5 sporangia ml^{-1}). Plants were fungicide-sprayed one and a half days after inoculation (timing determined according to Mills' curves and equivalent to 30% of the incubation period of *P. viticola*) with a manual glass sprayer using three QoI fungicides: azoxystrobin (Quadris 22.9% w:w a.i., Syngenta Crop Protection, Milan, Italy), trifloxystrobin (Flint 50% w:w a.i, Bayer CropSciences, Milan, Italy), famoxadone (dispersed in water, technical grade 100% w:w a.i., DuPont de Nemours, Cologno Monzese (MI), Italy). Fungicides were applied at the field rate (172, 125, 90 mg l^{-1} a.i.) and at double the field rate (343, 250, 180 mg l^{-1} a.i.).

Table 6. Sensitivity to Qo1 fungicides (expressed as percent leaf area showing downy mildew symptoms 15 days after the inoculation with 1×10^5 sporangia ml^{-1}) of populations of *P. viticola* collected in 2001 from vineyards located in Trentino Alto Adige and tested on potted grape plants.

Treatment	a.i. (mg l^{-1})	Population[a]										
		Reference[b]	5	7	8	10	11	13	14	16	18	35
Control	-	5.3 b[c]	1.4 ab	4.5 b	2.7 b	4.5 b	4.3 b	12.3 a	6.3 a	5.4 c	6.5 b	11.4 b
Azoxystrobin	172	0.0 a	1.5 ab	5.1 b	1.6 ab	1.7 ab	2.6 ab	20.9 a	0.2 a	1.1 ab	1.0 a	0.8 a
Azoxystrobin	343	0.4 a	2.7 b	2.6 ab	2.3 b	1.6 ab	1.9 ab	5.4 a	4.2 a	0.1 a	0.3 a	1.8 a
Trifloxystrobin	125	0.0 a	0.4 a	3.6 ab	1.8 ab	2.6 ab	2.1 ab	11.9 a	2.2 a	0.6 a	0.5 a	4.9 ab
Trifloxystrobin	250	0.1 a	1.1 a	1.9 ab	2.1 ab	0.5 ab	1.7 ab	15.2 a	0.6 a	0.8 ab	1.7 a	1.4 a
Famoxadone	90	0.0 a	0.5 a	3.1 ab	1.5 ab	2.2 ab	3.2 ab	11.7 a	1.8 a	2.4 b	0.8 a	6.7 ab
Famoxadone	180	0.4 a	0.4 a	1.9 ab	1.8 ab	2.8 ab	3.4 ab	6.8 a	0.7 a	1.4 ab	1.7 a	4.3 ab
Metalaxyl+ Mancozeb	10+160	0.4 a	0.0 a	0.5 a	0.2 a	0.0 a	0.1 a	5.5 a	0.4 a	0.1 a	0.0 a	1.7 ab

[a] The populations correspond to the ones tested in vitro with azoxystrobin (see Table 2).
[b] See Table 2.
[c] Values in the columns followed by the same letter are not statistically different by Tuckey's test ($P<0.05$).

As reference chemical, metalaxyl-M + mancozeb (Ridomil Gold MZ 46.2% w:w a.i., Syngenta Crop Protection) was used (10+160 mg l^{-1} a.i.). Three plants (30 leaves per plant) were used for each treatment.

Fifteen days after inoculation, the percentage of leaf area showing downy mildew was calculated. The experiment was carried out twice.

Statistical analysis

Data on the virulence of *P. viticola* from Trentino Alto Adige and Friuli Venezia Giulia (Table 5) resistant to QoI fungicides, and on the sensitivity of *P. viticola* to QoI fungicides in potted plants (Table 6) were subjected to analysis of variance. Mean values were separated with Tuckey's test.

Results

Leaf disc test

In vitro *evaluation of the sensitivity of* P. viticola *to QoI fungicides*

In 1999, in vineyards of Trentino Alto Adige, MIC values of the 21 tested populations ranged from 1.3 to 21.3 mg l^{-1} (Table 1).

In 2001, in the vineyards of Trentino Alto Adige, 5 to 100% of bunches inspected by the extension service were infected with downy mildew. MIC values were equal to or higher than 1000 mg l^{-1} in 21 samples out of 24, compared with 30 mg l^{-1} for the reference population (Table 2). In two vineyards where QoI had never been applied (No. 4 and 35), *P. viticola* populations did not differ from those sampled in QoI-treated vineyards. However, three cases of lower MIC values (100, 300) were obtained from vineyards never sprayed with QoI fungicides, or sprayed at a lower frequency (Table 2). All the vineyards monitored were located in fairly close proximity to each other.

In 2002, eight vineyards already sampled the previous year in Trentino Alto Adige were sampled again, plus an additional vineyard now sampled for the first time. All populations showed MIC values from 100 to >300 mg l^{-1}, while the reference population was completely inhibited at 10 mg l^{-1} (Table 2). None of the growers applied QoI fungicides in 2002.

In three of the four vineyards sampled in 2001 in Friuli Venezia Giulia, mildew incidence ranged from 60 to 90% . The MIC values were equal to or higher than 1000 mg l^{-1}, while the reference population was completely inhibited at 30 mg l^{-1} (Table 3).

In Piedmont, populations were sampled in vineyards where QoI fungicides had never been applied. In 2000 in this region MIC values of the 13 populations tested ranged from 1 to 30 mg l^{-1} compared with 30 mg l^{-1} for the reference population (Table 4). Disease incidence, expressed as percent infect-

ed leaves, ranged from <5 to 30%. Samples collected in Piedmont in 2002, with low to medium mildew incidence, showed MIC values from 0.3 to 3 mg l^{-1} (Table 4). Of the nine vineyards sampled in Trentino Alto Adige in 2002, eight had already been sampled in 2001 as well: populations of *P. viticola* in these vineyards all had much the same behaviour, with MIC values at least ten times that of the reference population (Table 2).

Evaluation of the sporulation capacity of populations of P. viticola

Populations of *P. viticola* collected in 2001 in Trentino Alto Adige and in Friuli Venezia Giulia, and resistant to QoI fungicides, showed variable virulence when increasing concentrations of sporangia (from 1.0×10^3 to 1.0×10^5) were inoculated on healthy leaves (Table 5). Some resistant populations, like nos. 3, 10 and 22 from Trentino Alto Adige and no. 4II from Friuli Venezia Giulia developed on the leaves even when the lowest sporangia concentration was inoculated. When 5.0×10^3 sporangia were inoculated, the number of resistant populations producing symptoms increased to 16 out of 30 (Table 5). Fifteen Trentino Alto Adige populations infected grapevine leaves when applied at 5×10^4 sporangia ml^{-1}. Only three populations (4, 29, 32) out of 22 (all characterised by resistance to QoI fungicides and all from Trentino Alto Adige) were unable to infect leaves when inoculated at a concentration of 1×10^5 sporangia ml^{-1} (Table 5).

Whole potted-plant test

All fungicides tested, including the reference mixture (metalaxyl-M+mancozeb), generally achieved complete or almost complete control of the sensitive population of *P. viticola*. Most populations that showed resistance to azoxystrobin in the leaf disc tests were not controlled by the QoI fungicides at field rates (172 mg l^{-1} of azoxystrobin, 125 mg l^{-1} of tryfloxystrobin and 90 mg l^{-1} of famoxadone). Double the field rate provided only partial control of downy mildew (Table 6).

Discussion

The intrinsic risk of resistance to QoI fungicides, due to their highly specific mode of action (Gisi, 2002), prompted the present monitoring study of vineyards in northern Italy to assess possible changes in QoI fungicide efficacy on grapevine. The sensitivity of *P. viticola* to QoI fungicides in Italy was monitored starting in 1999, one year after the registration of the first fungicide of this group, in Trentino Alto Adige, a region with a high incidence of downy mildew, and where chemical treatment tends to be frequent (Gullino *et al*., 1997). In 1999, resistance to QoI fungicides was not detected in any of the vineyards. Only two years later, however, in 2001, growers in Trentino Alto Adige started complaining about disease control failures in vineyards treated with QoI fungicides, and resistance was reported in commercial vineyards (Gullino *et al*., 2001).

QoI fungicide treatments were frequent in some vineyards in 2001 (6 in one case). This greatly exceeds the maximum number of sprays with QoI fungicides per year later recommended by FRAC (www.frac.info).

The results presented in this paper try to summarise and to give an overall idea of QoI fungicide resistance in some Italian vineyards, although it has to be stressed that nothing is known about the relative abundance of resistant individuals in the monitored fungal populations. Some difficulties also arose in manipulating a biotrophic pathogen like *P. viticola* (in particular storage and viability, as well as the handling of isolates).

The determination of a baseline type sensitivity to azoxystrobin for some populations of *P. viticola* collected in Trentino Alto Adige in 1999 provided a good reference standard for other populations subsequently collected in that and other areas (Friuli Venezia Giulia and Piedmont). The leaf discs assays on *P. viticola* confirmed the occurrence of populations with reduced sensitivity to QoI fungicides. QoI-resistant populations of *P. viticola* showed MIC values 10–30 times higher than that of the sensitive reference population in the same tests.

QoI-resistant populations of *P. viticola* were found in all the sampled vineyards in 2001 and 2002 in Trentino Alto Adige and Friuli Venezia Giulia where QoI fungicides had been widely applied. Many of the populations had MIC values 10–30 times those of the sensitive reference population.

In Piedmont, however, samples came from vineyards where QoI fungicides had not previously been used. In such vineyards, QoI compounds were still very effective, as shown by the MIC values of *P. viticola*.

Leaf disc test shows how some of the *P. viticola* populations retained their capacity to colonise grape leaves after various passages in water. Most of the QoI-resistant populations collected in Trentino Alto Adige in 2001 showed a significant capacity to infect grape leaves even at medium-low sporangia suspension concentrations (Table 5).

When QoI-resistant populations of *P. viticola* were tested on whole plants, all QoI fungicides tested showed a low efficacy against most of these populations, although the levels of infection were low. The failure to control mildew was evident also when these fungicides were applied at double the field rate. In some cases an almost complete inefficacy of QoI fungicides is shown, since values of percent leaf area showing downy mildew infection are close to or even higher than the untreated control values.

The occurrence of QoI-resistant *P. viticola* populations in vineyards that had never been sprayed with this class of fungicides, but that were adjacent to sprayed vineyards, indicates that disease management must be regional rather than strictly local. A lower sensitivity to QoI fungicides in many cases, coupled with cases of disease control failure in vineyards treated with such fungicides and the ability of less sensitive populations of *P. viticola* to infect leaves treated with QoI at a dosage that controls a more sensitive population, leads to the conclusion that field resistance has developed and that QoI fungicides must be applied in strict accordance with the recommendations of FRAC. This means that, in order to maintain their efficacy at a high level, a limited and careful use must be made of all fungicides that show cross-resistance (i.e. azoxystrobin, kresoxim methyl, trifloxystrobin, famoxadone, fenamidone) and that they should be alternated with compounds having a different mode of action. Finally, on grapevine, a maximum of 3 applications of QoI/season is now recommended by FRAC.

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