Eur J Plant Pathol (2011) 129:331–338
DOI 10.1007/s10658-010-9677-y

Evolution of QoI resistance in *Plasmopara viticola* oospores

Silvia Laura Toffolatti · Marisol Prandato · Luca Serrati · Helge Sierotzki · Ulrich Gisi · Annamaria Vercesi

Accepted: 23 August 2010 / Published online: 9 September 2010

Abstract QoI resistance in *P. viticola* was first detected in France and Italy in 1999. Molecular and biological assays have been carried out since 2000 in order to provide reliable methods of detecting and quantifying resistance. Oospores were collected in vineyards located in northern and southern Italy. QoI resistance was evaluated by the germination rate of oospores on azoxystrobin amended medium and the frequency of mutant alleles in the DNA extracted from oospores. Both methods correlated to each other and were used side by side to test QoI resistance. Due to the spontaneous occurrence of the G143A mutation in wild type populations and the immigration from surrounding vineyards, resistance frequencies up to 10% were found in samples collected from vineyards never treated with QoIs. Particularly high values, about 90%, were associated with the application of five to six QoI treatments within the same season, while lower percentages, about 30%, were detected in vineyards treated with QoI used in mixture with fungicides belonging to a different resistance group. A progressive decrease of resistance frequency was observed when QoI applications were reduced in number or completely suspended for at least one season. Therefore, a full recovery of sensitivity may be achieved even in vineyards characterized by high levels of resistance, if particular care is taken during disease control by using QoIs only in mixtures and reducing the number of QoI treatments.

Keywords Downy mildew · Fungicide resistance · Grapevine · Resistance management · Sensitivity assay

S. L. Toffolatti (✉) · M. Prandato · A. Vercesi
Facoltà di Agraria, Di.Pro.Ve.
Sezione di Patologia Vegetale,
Università degli Studi di Milano,
via Celoria 2,
20133 Milano, Italy
e-mail: silvia.toffolatti@unimi.it

L. Serrati
Development Center South East Europe,
Syngenta Crop Protection,
via Gallarate 139,
20151 Milano, Italy

H. Sierotzki · U. Gisi
Research Biology, Syngenta Crop Protection,
WST-540, Schaffhauserstrasse,
4332 Stein, Switzerland

Introduction

Grapevine downy mildew caused by *Plasmopara viticola* (Berk. *et* Curt.) Berl. and De Toni is a serious disease in climates characterized by moderate temperatures and frequent rainfall in spring and summer (Gilles 2004; Jeger and Pautasso 2008). These conditions occur in different viticultural area and years leading to severe epidemics, as it was observed in the past few years first in southern and then in northern Italy. Chemical control is currently the most effective measure to protect the plants from disease. Numerous treatments with fungi-

cides are carried out in vineyards during the grapevine growing season to prevent severe epidemics that can reduce both yield and quality of grapes. Due to its biological features (Burruano 2000; Lafon et al. 1988), *P. viticola* is considered as a high risk pathogen for resistance developing to fungicides (FRAC pathogen risk list, www.frac.info). It is therefore necessary to avoid high selection pressure on populations, which may favour the appearance of resistant strains. In order to prevent failures in disease control and to preserve the performance of products, it is necessary to optimize the use of all fungicide classes available against grapevine downy mildew.

Among the fungicides most frequently used in vineyards, QoI fungicides such as azoxystrobin, famoxadone and fenamidone are important (Gisi and Sierotzki 2008). All QoIs are sharing the same target site since they all inhibit mitochondrial respiration by preventing binding and subsequent oxidization of hydroquinone at the Quinol outer (Qo) site of cytochrome bc1 complex (Link et al. 2003; Kuck and Gisi 2007). Because of the single-site mode of action of QoIs and their extensive use, resistant strains in *P. viticola* population were detected already in 1999, first in field trials in Italy and France and subsequently in commercial vineyards (Bartlett et al. 2002). Monitoring methods are commonly used to evaluate the frequency of resistant strains and the effect of different strategies of fungicide application on pathogen populations (Brent and Hollomon 1998).

Since the discovery that QoI resistance in *P. viticola* correlates with a single nucleotide polymorphism in the cytochrome b gene leading to an amino acid change at position 143 (G143A) (Sierotzki et al. 2005; Chen et al. 2007), molecular methods allow a fast and accurate detection of QoI resistance by an allele-specific, real-time PCR assay (Sierotzki and Gisi 2003). The possibility of using this highly sensitive method improved the possibility of monitoring the spread of resistant strains and studying the evolution of fungicide resistance at the population level (Ma and Michailides 2005; Sierotzki et al. 2005). In general, QoI sensitivity can be monitored by collecting *P. viticola* strains in vineyards and evaluating their sporulation on leaf discs treated with different fungicide concentrations (www.frac.info) or by molecular methods (Sierotzki and Gisi 2003).

Biological and molecular assays have also been carried out by using oospores (Vercesi et al. 2002; Toffolatti et al. 2007). The sexual structures of the pathogen are differentiated in the infected leaves at the end of the grapevine growing season. They overwinter and germinate the following season, providing inoculum for primary infections (Vercesi et al. 2000). QoI resistance is inherited maternally (Gisi et al. 2002); therefore, QoI resistance can be studied in oospores collected at the end of the grapevine growing season, reflecting the result of the selection imposed by the fungicide applications made during the past season, and indicating the potential frequency of resistance in the primary inoculum for the following season.

In this paper, the evolution of resistance was investigated in vineyards which received either no QoI treatments or were treated with solo formulations or mixtures with non-cross resistant fungicides. The results support a more rational rather than routine use of these fungicides.

Materials and methods

Sampling

Leaves showing downy mildew symptoms were randomly collected during the seasons 2004 to 2007 in 33 vineyards located mainly in southern Italy (Puglia) but also in northern Italy (Lombardia, Veneto, Piemonte and Emilia Romagna). In particular, 7 vineyards were monitored for at least 2 consecutive years in order to follow the evolution of QoI resistance after QoI use suspension (Tables 1, 3): in the two vineyards located in Emilia Romagna, two adjacent plots, respectively untreated (3P and SG) and treated with QoI fungicides (3PT and SGT) were sampled. A single leaf fragment (0.5 cm^2), containing a high number of oospores (as determined microscopically with Leitz Orthoplan), was cut from each leaf. Fifty leaf fragments per replicate were transferred to nylon bags (pore diameter 100 μm) and overwintered at 5°C in the dark laying on a sand substrate kept water-saturated. Four replicates were prepared per sampling location.

Biological and molecular assays

The biological assay, based on the germination rates of oospores on azoxystrobin amended medium at the

Table 1 Origin of samples and average rates of RO and MA

Sample	Region	Year of sampling	QoI treatment	RO (%)	MA (%)
BAE	Puglia	2004	solo	81	95
BAE	Puglia	2005	solo	36	48
BAE	Puglia	2006	solo	85	76
BAA	Puglia	2004	solo	12	19
BAD	Puglia	2004	solo	97	100
BAS	Puglia	2004	solo	94	98
BAG	Puglia	2004	solo	74	76
BAF	Puglia	2004	solo	94	98
BAF	Puglia	2006	solo	70	93
BAZ	Puglia	2004	solo	31	18
BAB	Puglia	2004	solo	79	85
BAN	Puglia	2004	solo	1	10
BAP	Puglia	2004	solo	14	70
BAU	Puglia	2004	solo	52	83
BAO	Puglia	2004	solo	87	98
BAM	Puglia	2004	solo	20	24
BBC	Puglia	2004	solo	0,4	1
BBI	Puglia	2005	solo	22	29
BBL	Puglia	2005	solo	28	39
BBL	Puglia	2006	solo	66	43
MO	Piemonte	2005	solo	13	11
MO	Piemonte	2006	solo	0	0.5
BAH	Puglia	2004	mixture	0	0
BAB	Puglia	2005	mixture	21	31
BBH	Puglia	2005	mixture	72	71
BBH	Puglia	2006	mixture	55	98
BBM	Puglia	2006	mixture	10	15
BAT	Puglia	2004	mixture	16	22
BAR	Puglia	2004	mixture	0	0
BBN	Puglia	2006	mixture	24	47
SO	Lombardia	2006	mixture	42	36
BAF	Puglia	2005	no QoI	10	8
BAM	Puglia	2005	no QoI	25	25
BAI	Puglia	2005	no QoI	0	1
BAI	Puglia	2006	no QoI	11	16
BAI	Puglia	2007	no QoI	0	9
BBI	Puglia	2006	no QoI	2,6	5
BBD	Puglia	2004	no QoI	3,1	7
MT	Veneto	2004	no QoI	5	9
MT	Veneto	2005	no QoI	0	10
MT	Veneto	2006	no QoI	13	20
MS	Veneto	2005	no QoI	6	8
MS	Veneto	2006	no QoI	4	11
BE	Lombardia	2005	no QoI	12	0.5
BE	Lombardia	2006	no QoI	2	3

discriminatory dose of 10 mg l^{-1}, was carried out every year at the end of January as described by Toffolatti et al. (2007). Oospore germination (1200 oospores per sample) was assessed on 1% water agar (Agar Noble, Difco) with and without 10 mg l^{-1} of azoxystrobin (technical grade) at 20°C. Percentage of oospore germination (G) was determined by counting the number of germinated structures, which was checked daily over a period of 14 days under the microscope (Leica Wild M10). The rate of resistant oospores (RO) was calculated as Gaz×100/Gwa, where Gaz and Gaw are the germination rates of the oospores on water agar amended with azoxystrobin and on water agar.

The molecular assay was carried out according to Toffolatti et al. (2007, 2008) by allele specific detection and quantification in real-time PCR of the SNP leading to G143A amino acid substitution in cytochrome b protein. Quantification of both mutated and wild type alleles in the DNA extracted from the oospores (CTAB method) was performed on ABI prism 7000 thermal cycler (Applied Biosystems, USA) by using ARMS-Scorpion or SYBR Green detection methods. Double distilled water and DNA extracted from the leaves were used as controls in order to detect possible unspecific amplifications. The efficiency of the PCR assay was estimated from standard curves based on serial dilutions of purified plasmids containing the target sequences.

The frequency of mutated alleles ([1/(r+1)]×100) was calculated from the ratio (r) of both alleles as $r=2^{\Delta Ct}$, where $\Delta C_t=C_t$mut-$C_t$wt and Ct are the threshold cycle values (C_t) for G143 (wt) and 143A (mut) alleles.

Statistical analysis

Shapiro-Wilk's test for normality was used to evaluate the distribution of the mean frequency of resistant oospores (RO) and mutated allele 143A (MA). Non-parametric correlation between the two variables was assessed by Spearman's R and Kendall's Tau coefficients. Differences between samples collected from vineyards with different use strategies of QoI's (solo, in mixture with partner belonging to another resistance group, and not applied at all) were visualized by box-plots. Box-plots visualise the variability existing among populations even if the underlying statistical distributions are unknown. They show the shape, characteristic values and variability of a distribution by using the maximum and minimum values ("whiskers"), the interquartile range (i.e. the difference between the third and first interquartile), IQR (box), and the median (central line) of the data set. One-way ANOVA and *a priori* contrasts, tested with t statistics, were performed on transformed RO and MA ($\arcsin\sqrt{x/100}$) with the aim of determining differences in the mean values of different treated samples. Statistical analysis was carried out with the SPSS Statistics 17.0 software.

Results

Frequency of resistant oospores and mutated allele

Since the hypothesis of a normal distribution was rejected following the results of Shapiro-Wilk's test on the mean frequency of RO (W=0.83, p=0.000) and MA (W=0.84,

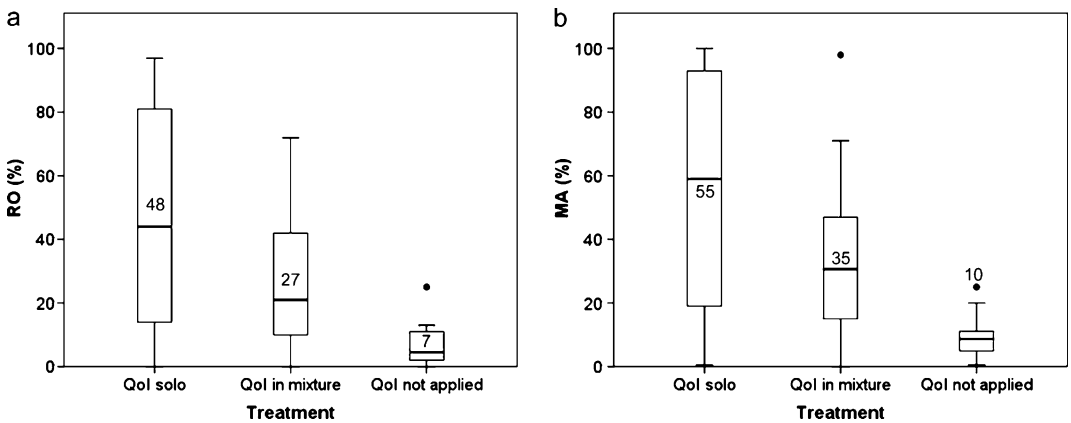

Fig. 1 Boxplot analysis of RO (**a**) and MA (**b**) following different strategies of QoI treatment. Numbers indicate mean values, *black circles* represent mild outliers

Table 2 *A priori* sets and values of contrasts on transformed values of RO and MA and results of *t*-test

	Contrast	Value of contrast	Standard error	*t*	df[a]	Significance
RO	Solo+mixtures against untreated	0.7791	0.23536	3.310	42	0.002
	Solo against untreated	0.5254	0.12099	4.342	42	0.000
	Mixture against untreated	0.2538	0.15120	1.678	42	0.101
	Solo against mixture	0.2716	0.14003	1.940	42	0.059
MA	Solo+mixtures against untreated	0.8591	0.25974	3.308	42	0.002
	Solo against untreated	0.5647	0.13353	4.229	42	0.000
	Mixture against untreated	0.2945	0.16687	1.765	42	0.085
	Solo against mixture	0.2702	0.15454	1.749	42	0.088

[a] Degrees of freedom

p=0.000), Spearman's and Kendall's coefficients for non-parametric correlation between variables were calculated. A strong linear and positive correlation between the values of MA and RO (R=0.93, *Tau*=0.80) was found, showing that both parameters can be used to estimate QoI resistance in *P. viticola* oospores.

Effect of QoI treatments

The application of QoIs as solo formulations resulted in high variability in the rates of both RO and MA, which ranged from 0 to 100% with an IRQ of 70% and a non-normal distribution of the samples (W=0.89, p=0.019

Table 3 Origin of samples collected from vineyards where treatments with QoI fungicides were suspended and average RO

Sample	Region	Year of sampling	QoI treatment	RO (%)
3P	Emilia Romagna	2001	no QoI	98
3P	Emilia Romagna	2002	no QoI	18
3P	Emilia Romagna	2003	no QoI	33
3P	Emilia Romagna	2004	no QoI	17
3P	Emilia Romagna	2005	no QoI	20
3P	Emilia Romagna	2006	no QoI	27
3PT	Emilia Romagna	2001	solo	93
3PT	Emilia Romagna	2004	no QoI	17
3PT	Emilia Romagna	2005	no QoI	16
3PT	Emilia Romagna	2006	no QoI	4
SG	Emilia Romagna	2002	no QoI	72
SG	Emilia Romagna	2004	no QoI	69
SG	Emilia Romagna	2005	no QoI	17
SG	Emilia Romagna	2006	no QoI	17
SGT	Emilia Romagna	2002	mixture	86
SGT	Emilia Romagna	2004	no QoI	42
SGT	Emilia Romagna	2005	no QoI	14
SGT	Emilia Romagna	2006	no QoI	9
BAF	Puglia	2005	solo	90
BAF	Puglia	2006	no QoI	10
BAM	Puglia	2004	solo	22
BAM	Puglia	2005	no QoI	25
BAM	Puglia	2006	no QoI	27
BBI	Puglia	2005	mixture	22
BBI	Puglia	2006	no QoI	3

for RO, W=0.88, p=0.013 for MA). Despite the mean values of RO and MA (48 and 55%, respectively, Fig. 1), the samples can be divided into two main groups: the first and the larger one is characterized by low (0–50%) and the second by high (70–100%) rates of resistance (Table 1).

The box-plots showed reduced median resistance values and decreasing variation (IQR) in the samples collected from vineyards where QoIs were applied in mixture: for both indices measured, RO and MA, the 50% portion of the examined samples ranged from 10 to 40–45%, and the mean values were 27 and 35%, respectively (Fig. 1). Particularly low mean values and IQR, about 10%, were detected for oospores collected in vineyards which were not treated with QoIs at all (Fig. 1). In addition, the extreme values were very close to the median in the untreated samples, indicating a narrow distribution of the data.

Statistically significant differences were found between the means of samples with different treatments: F=9.558, $P(F)$=0.000 for RO and F=9, $P(F)$=0.000 for MA. *A priori* contrasts showed differences between treated (solo and mixtures) and untreated samples and between solo treated and untreated samples, whereas samples treated in mixture did not differ from solo nor untreated (Table 2).

Effect of QoI use suspension

In seven vineyards samples were collected in subsequent years in order to assess the evolution of resistance following QoI use suspension (Table 3). QoI sprays in the first year of monitoring resulted in a mean RO of 63%, but 25% of the population showed particularly high rates, ranging between 85% and 95% (Fig. 2). In Emilia Romagna, similar rates of resistant oospores were found in the untreated plots, 3P and SG, located near the QoI treated ones. Despite the wide range of RO observed in the first year since QoI suspension, much smaller values were observed for IQR (15–55%) and the median (25%), indicating a shift in the pathogen population towards reduced resistance (Fig. 2). Two years after suspension, RO showed a positively skewed distribution with a reduced IQR, ranging between 15% and 30%, and a median value of 18%. At the end of the third year, the number of resistant oospores further decreased towards values similar to those observed in vineyards where QoI were not applied (mean 16%, median 13%).

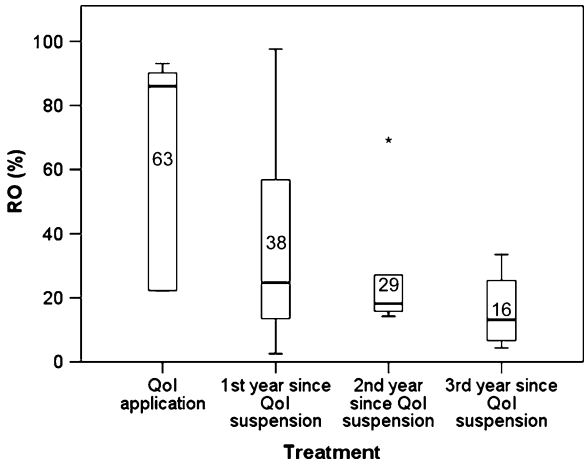

Fig. 2 Box-plot analysis of RO in samples collected from vineyards where QoI sprays were suspended. Numbers indicate mean values, asterisks extreme outliers

Discussion

Since a large number of oospores, each representing a single individual, can be easily collected in many vineyards, detection of *P. viticola* resistance to QoI fungicides using the oospore method is highly representative for entire populations and can be particularly useful in base-line sensitivity assays. Moreover, rapid and quantitative detection of resistant strains in pathogen populations enables the prediction of the resistance risk and may prevent control failure by using adapted fungicide use strategies (Ishii 2006; Macdonald 2008).

Since the discovery of resistance, numerous studies have been carried out on both the underlying mechanism and inheritance (Blum and Gisi 2008; Gisi et al. 2002; Grasso et al. 2006). Phylogenetic analysis of a large mitochondrial DNA fragment including cytochrome *b* gene sequences of different strains of *P. viticola* showed that QoI resistance emerged independently several times in Europe (Chen et al. 2007). The strong correlation between the rates of RO and MA confirms that QoI resistance is mainly based on the point mutation in the cytochrome b gene leading to the amino acid substitution G143A (Sierotzki et al. 2005; Toffolatti et al. 2007; Chen et al. 2007).

The effect of the different QoI treatments (singly and in mixture) on pathogen sensitivity was evaluated by analysing the distribution of RO and MA by ANOVA and *a priori* contrasts on the transformed data. The repeated application of QoI fungicides, especially as a solo formulation, which

was done in past years, significantly increased the initially low proportion of resistance of *P. viticola* populations from 0% to 20% to variable but large values (0% to 100%), not only in the directly treated plots, but also in the untreated vineyards parts located nearby, due to the migration of the pathogen strains (Toffolatti et al. 2008). Moreover, solo treatments induced a separation of the samples in two subpopulations, characterized by low (about 15%) and high (about 80%) rates of resistance. Due to sexual recombination and migration of sensitive strains from surrounding vineyards, a high selection pressure does not always result in oospore populations with high resistance rates. The samples collected from vineyards following the utilization of QoI in mixture with fungicides belonging to different resistance groups, such as inhibitors of RNA synthesis (phenylamide fungicides) or multi-site inhibitors such as folpet, showed reduced frequencies of resistance, compared to solo applications, confirming the lower selection pressure of this use strategy (Russell 2003; Genet et al. 2006).

A shift towards low resistance levels similar to those in untreated vineyards was observed following the interruption of QoI sprays for at least 2 years. A substantial reduction in the distribution of resistance was found starting from the first year of interruption, followed by a further recovery of sensitivity starting from the second year onwards.

Studies carried out with microsatellite markers suggested that QoI resistant strains of *P. viticola* possess fitness properties as high as sensitive strains during asexual cycles *in vitro* and *in natura* (Corio-Costet et al. 2008; Lafarge et al. 2008). However, a decline in the frequency of resistant strains of *P. viticola* was observed after stopping QoI treatments (Gisi and Sierotzki 2008). Also in the present study, we demonstrated that resistance in oospore populations decreases when selection pressure is reduced. Therefore, a more sensitive primary inoculum can be expected for the next grapevine season, if fungicide mixtures instead of solo formulations are used or, in cases of high resistance frequency, if QoI treatments are completely suspended for at least 2 years.

The presented data suggest that usage of QoIs in mixture is a suitable strategy to manage resistance. The data suggest that while QoIs cannot be totally excluded, their usage in mixtures is a suitable strategy to manage resistance. However, it is necessary that the frequency of resistant strains decreases to a low level in the population and that the population is monitored continuously for changes.

The preservation of many fungicides is more and more critical since the application of the Directive 91/414/EEC which will be replaced by the recent regulation (EC) No 1107/2009 of 21 October 2009, concerning the placement of plant protection products on the market in the European Community, and of the Directive 99/45/EC, related to the classification, packaging and labelling of dangerous preparations. Therefore, the number of fungicides available on the market could be reduced. As a consequence, it is very important to maintain as many fungicide classes as possible through sound resistance monitoring and use strategies.

References

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., & Parr-Dobrzanski, B. (2002). The strobilurin fungicides. *Pest Management Science, 58*, 649–662.

Blum, M., & Gisi, U. (2008). Inheritance of fungicide resistance in *Plasmopara viticola*. In H. W. Dehne, H. B. Deising, U. Gisi, K. H. Kuck, P. E. Russel, & H. Lyr (Eds.), *Modern fungicides and antifungal compounds V* (pp. 101–104). Braunschweig: DPG Selbstverlag.

Brent, K. J., & Hollomon, D. W. (1998). *Fungicide resistance: The assessment of risk*. Brussels: Global Crop Protection Federation.

Burruano, S. (2000). The life-cycle of *Plasmopara viticola*, cause of downy mildew of vine. *Mycologist, 14*, 179–182.

Chen, W. J., Delmotte, F., Richard-Cervera, S., Douence, L., Greif, C., & Corio-Costet, M. F. (2007). At least two origins of fungicide resistance in grapevine downy mildew populations. *Applied and Environmental Microbiology, 73*, 5162–5172.

Corio-Costet, M. F., Martinez, F., Delmotte, F., Douence, L., Richart-Cervera, S., & Chen, W. J. (2008). Resistance of *Plasmopara viticola* to QoIs fungicides: Origin and diversity. In H. W. Dehne, H. B. Deising, U. Gisi, K. H. Kuck, P. E. Russel, & H. Lyr (Eds.), *Modern fungicides and antifungal compounds V* (pp. 107–112). Braunschweig: DPG Selbstverlag.

Genet, J. L., Jaworska, G., & Deparis, F. (2006). Effect of dose rate and mixtures of fungicides on selection for QoI resistance in populations of *Plasmopara viticola*. *Pest Management Science, 62*, 188–194.

Gilles, T. (2004). Forecasting downy mildew disease. In P. Spencer-Phillips & M. Jeger (Eds.), *Advances in downy mildew research* (Vol. 2, pp. 35–67). Dordrecht: Kluwer Academic.

Gisi, U., & Sierotzki, H. (2008). Fungicide modes of action and resistance in downy mildews. *European Journal of Plant Pathology, 122*, 157–167.

Gisi, U., Sierotzki, H., Cook, A., & McCaffery, A. (2002). Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. *Pest Management Science, 58*, 859–867.

Grasso, V., Palermo, S., Sierotzki, H., Garibaldi, A., & Gisi, U. (2006). Cytochrome b gene structure and consequences for resistance to QoI inhibitor fungicides in plant pathogens. *Pest Management Science, 62*, 465–472.

Ishii, H. (2006). Impact of fungicide resistance in plant pathogens on crop disease control and agricultural environment. *Japan Agricultural Research Quarterly, 40*, 205–211.

Jeger, M. J., & Pautasso, M. (2008). Comparative epidemiology of zoosporic plant pathogens. *European Journal of Plant Pathology, 122*, 111–126.

Kuck, K.-H., & Gisi, U. (2007). FRAC mode of action classification and resistance risk of fungicides. In W. Krämer & U. Schirmer (Eds.), *Modern crop protection compounds* (pp. 415–432). Weinheim: Wiley-VCH.

Lafarge, D., Cigna, J., Abadie, P., Douence, L., Dufour, M. C., & Corio-Costet, M.-F. (2008). Competitive fitness and adaptative value of QoI-resistant *Plasmopara viticola* strains. In H. W. Dehne, H. B. Deising, U. Gisi, K. H. Kuck, P. E. Russel, & H. Lyr (Eds.), *Modern fungicides and antifungal compounds V* (pp. 181–185). Braunschweig: DPG Selbstverlag.

Lafon, R., Clerjeau, M., Pearson, R. C., & Goheen, A. C. (1988). Downy mildew. In R. C. Pearson & A. C. Gohen (Eds.), *Compendium of grape diseases* (pp. 11–13). St. Paul: APS Press.

Link, T. A., Iwata, M., Björkman, J., van der Spoel, D., Stocker, A., & Iwata, S. (2003). Molecular modeling of inhibitors at Qi and Qo sites in cytochrome bc1 complex. In G. Voss & G. Ramos (Eds.), *Chemistry of crop protection* (pp. 110–127). Weinheim: Wiley.

Ma, Z., & Michailides, T. J. (2005). Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Protection, 24*, 853–863.

Macdonald, O. C. (2008). Regulatory aspects of resistance management. In H. W. Dehne, U. Gisi, K. H. Kuck, P. E. Russell, & H. Lyr (Eds.), *Modern fungicides and antifungal compounds V* (pp. 113–120). Alton: BCPC.

Russell, P. (2003). Taking the path of least resistance. *Pesticide Outlook, 14*, 57–61.

Sierotzki, H., & Gisi, U. (2003). Molecular diagnostics for fungicide resistance in plant pathogens. In G. Voss & G. Ramos (Eds.), *Chemistry of crop protection* (pp. 71–88). Weinheim: Wiley.

Sierotzki, H., Kraus, N., Assemat, P., Stanger, C., Cleere, S., Windass, J., et al. (2005). Evolution of resistance to QoI fungicides in *Plasmopara viticola* populations in Europe. In H. W. Dehne, U. Gisi, K. H. Kuck, P. E. Russell, & H. Lyr (Eds.), *Modern fungicides and antifungal compounds IV* (pp. 73–80). Alton: BCPC.

Toffolatti, S. L., Serrati, L., Sierotzki, H., Gisi, U., & Vercesi, A. (2007). Assessment of QoI resistance in *Plasmopara viticola* oospores. *Pest Management Science, 63*, 194–201.

Toffolatti, S. L., Prandato, M., Serrati, L., Sierotzki, H., Gisi, U., & Vercesi, A. (2008). Monitoring QoI resistance in *Plasmopara viticola* oospore populations. In H. W. Dehne, H. B. Deising, U. Gisi, K. H. Kuck, P. E. Russel, & H. Lyr (Eds.), *Modern fungicides and antifungal compounds V* (pp. 159–165). Braunschweig: DPG Selbstverlag.

Vercesi, A., Sirtori, C., Vavassori, A., Setti, E., & Liberati, D. (2000). Estimating germinability of *Plasmopara viticola* oospores by means of neural networks. *Medical & Biological Engineering & Computing, 38*, 109–112.

Vercesi, A., Vavassori, A., Faoro, F., & Bisiach, M. (2002). Effect of azoxystrobin on the oospores of *Plasmopara viticola*. In P. T. N. Spencer-Phillips, U. Gisi, & A. Lebeda (Eds.), *Advances in downy mildew research* (pp. 195–199). Dordrecht: Kluwer Academic.