15
Resistance of *Plasmopara viticola* to QoI Fungicides: Origin and Diversity

M.-F. CORIO-COSTET[1], F. MARTINEZ[2], F. DELMOTTE[1], L. DOUENCE[1], S. RICHART-CERVERA[1] and W.J. CHEN[1]
[1]*INRA, UMR Santé Végétale, ISVV, IFR 103, 71 Avenue E. Bourlaux, BP81 33883 Villenave d'Ornon Cedex, France*
[2]*ITV-Bordeaux, Blanquefort, France*

Abstract
The effectiveness of QoI fungicides against grape downy mildews in European vineyards has decreased significantly in the last years. A single nucleotide polymorphism, G143A in the cytochrome *b* gene of *Plasmopara viticola* was detected to confer QoI resistance. Polymorphism analyses of the mitochondrial genome showed that 4 major haplotypes (IR, IS, IIR, IIS) coexisted in French vinyards. By contrast, mitochondrial and RNA 28s analyses showed that 3 sub-species coexisted in vineyards of the USA. In Bordeaux vineyards, the most frequent haplotype (IR, IS) in *P. viticola* population reached 74%. The resistant allele frequencies ranged from 0 to 75 % with an average of 29 %. Therefore, at least two independent events led to the emergence of QoI resistance. By combining (non-coding) microsatellite and selective markers, a temporal genetic structure was obtained for *P. viticola* populations in which genetic variability was low and genotypic richness was high. To manage QoI resistance, it is important to understand how resistant populations appear, spread and survive.

Introduction

Plasmopara viticola, the causal agent of grapevine downy mildew, is a native species of North America that was introduced into Europe in 1878. For effective control, QoI fungicides have been used in France since 1998 but only two years later, resistant populations were detected in French vineyards (Magnien *et al.*, 2003). The selection of well characterized mutations associated with QoI resistance provides the opportunity to understand the appearance, spread and survival of downy mildew populations. In most pathogens, resistance to QoIs is conferred by a major point mutation in the mitochondrial cytochrome *b* gene giving rise to a substitution from glycine to alanine at codon 143 (G143A) (Gisi *et al.*, 2002; Grasso *et al.*, 2006; Chen *et al.*, 2007). In this investigation, we assessed the evolution of QoI resistance using mitochondrial sequence analysis of the complete cytochrome *b* gene of a broad range of sensitive and resistant isolates of *P. viticola*. The following questions were asked: 1) How many mutations are involved in QoI resistance? 2) Does diversity of *P. viticola* populations

Modern Fungicides and Antifungal Compounds V
 - ISBN 978-3-941261-02-0

differ with regard to their level of resistance? 3) What is the distribution of QoI resistant haplotypes in the Bordeaux vineyard? 4) What is the genetic structure of QoI-resistant populations in different locations?

Material and Methods

A total of 839 isolates were collected in 2003 at the beginning of the growing season from 23 locations in Bordeaux vineyards which were not treated with QoIs. In addition, fourteen isolates coming from the Finger Lake region in the USA and 56 from Europe were used for phylogenetic analysis. Another 513 isolates were sampled in 2004 at three locations in the Bordeaux area on two dates (Bomme: June 22nd, September 1st; Latresne: June 15th, August 6th; Blanquefort: June 23rd, September 1st) and analysed for genetic structure of the population.

Mitrochondrial markers

A 2281 bp fragment of mitochondrial genome including cytochrome *b* gene was sequenced revealing four mitochondrial haplotypes (IS, IR, IIS, IIR, Chen *et al.*, 2007). Haplotype identification was performed by the CAPS method. After PCR amplification, DNA was digested by two restriction enzymes *Sat1* for detecting resitant haplotypes (IR, IIR) and *Hinfl* to identify haplotypes I and II (Corio-Costet *et al.*, 2006).

Microsatellite markers

Eight microsatellites were used as described by Delmotte *et al.* (2006) to determine genetic variability. Allelic frequency and fixation index (Fis: fixation index in each population) were calculated with GENEPOP (Raymond & Rousset, 1995). The genetic structure was examined by analysis of molecular variance (AMOVA).

Results

Diversity and phylogeny

Based on a fragment of *P. viticola* mitochondrial DNA including two complete genes (cytochrome *b* and NAD9), two partial genes (ATP9 and NAD5) and three intergenic regions were characterized for the four major mitochondrial haplotypes in European isolates (Figure 1). Two resistant haplotypes (IR, IIR) were detected exhibiting a mutation at codon 143 (G143A) and two sensitive haplotypes (IS, IIS) exhibiting a group of 6 linked mutations on the fragment.

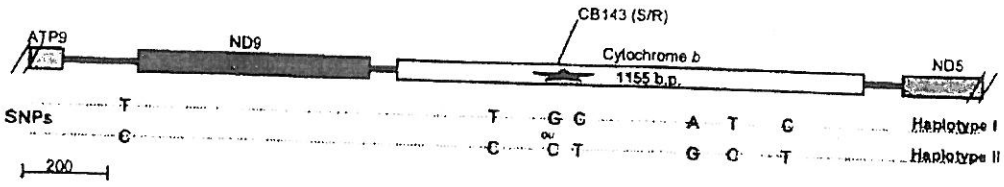

Figure 1. Schematic representation of different mutations identifying the four major haplotypes in European populations of *Plasmopara viticola*

Phylogeny analyses based on mitochondrial and RNAr 28S variabilities from 56 European and 14 American isolates showed a high variability of cytochrome *b* alleles in the American isolates. RNAr 28S analyses suggested that at least 3 new sub-species of *Plasmopara* on grapevine can be distinguished in the USA (Figure 2).

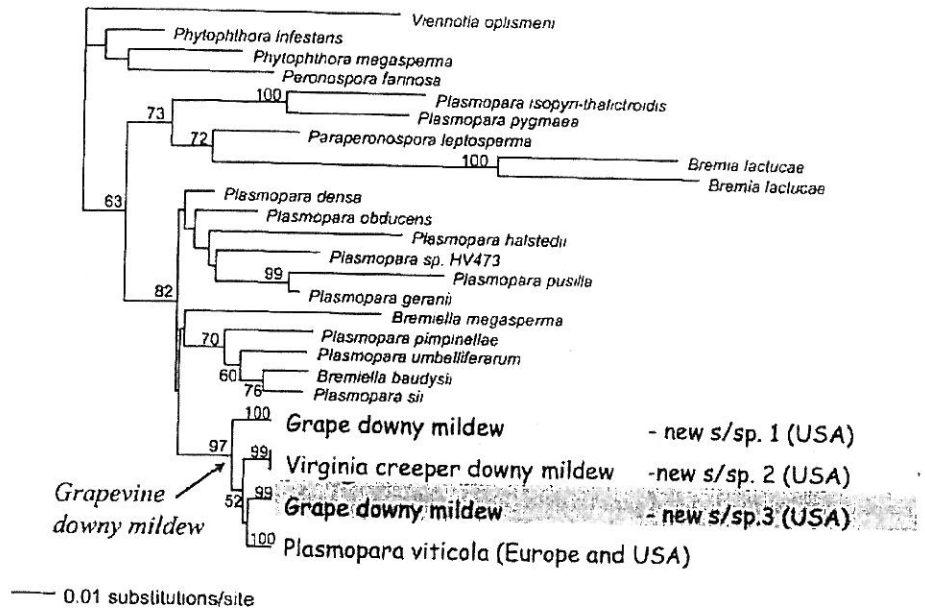

Figure 2. Phylogenetic tree based on polymerphism of RNAr 28S of different oomycetes and European and American isolates of *Plasmopara viticola*.

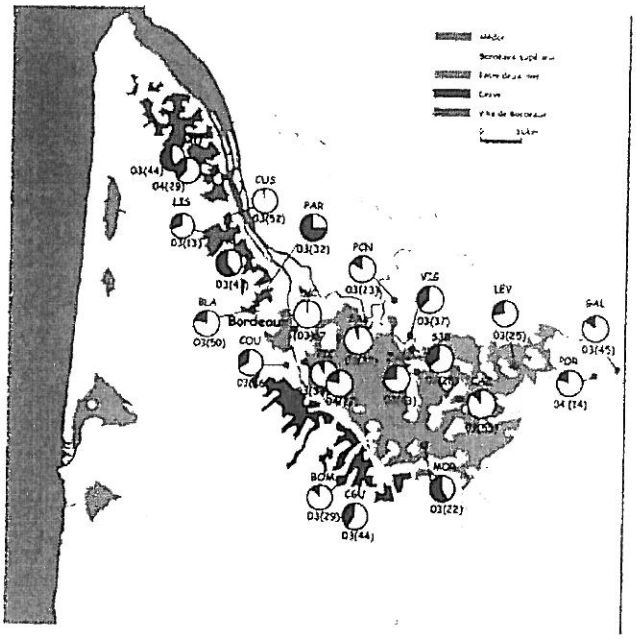

Figure 3. Distribution of sensitive (white) and QoI resistant (black) alleles of *Plasmopara viticola* cytochrome *b* in 23 locations of Bordeaux vineyard in 2003 (number of isolates in paranthesis).

Distribution of QoI resistance and genetic structure

A total of 839 isolates were used for evaluating resistance and haplotype frequencies in *P. viticola* populations of the Bordeaux area in 2003. Haplotype I (IR, IS) reached a frequency of 74% and haplotype II (IIR, IIS) 25%. The percentage of resistant alleles ranged from low (0) to very high (75) (Figure 3) with an average of 29.9%. In resistant populations, haplotype I represented 85.8% and haplotype II 14.2%.

By using three microsatellite markers, the genotypic diversity of *P. viticola* populations in 2004 was found to be 0.73. Resistant populations with a resistant allele frequency > 30% tended to exhibit a low genotypic diversity ($R^2 = 0.54$) suggesting a high asexual multiplication in these populations. By combining microsatellite markers (8) and selected markers, the temporal genetic structure of *P. viticola* populations was characterized at three localities on two dates in 2004. The level of QoI resistance was low (0 to 16%, Table 1). In untreated plots the frequency of haplotype II ranged from 9.3 to 31.2%. No significant variation in frequency was observed between the two dates except that haplotype II disappeared in the Blanquefort plot at the second date of sampling (Table 1). The genotypic diversity (G/N) decreased between the two dates in Blanquefort and Bommes plots suggesting high asexual multiplication.

Table 1. Haplotype distribution and genetic data (Fis) measured in each population for each microsatellite locus. (*) significant result.

		N	G/N	% haplotypes				% resistance to QoIs	Fis
				I		II			
				SI	RI	SII	RII		
Blanquefort	date 1	71	0.56	66.4	5.9	25.2	2.5	8.9	0.157*
	date 2	111	0.21	91.7	6.5	1.4	0.4	16.0	-0.094
Bommes	date 1	39	0.74	67.1	3.7	29.2	0	6.9	-0.085
	date 2	45	0.53	68.8	0	31.2	0	8.4	-0.016
Latresne	date 1	127	0.83	79.8	10.9	9.3	0	0	0.033*
	date 2	120	0.70	78.0	9.3	12.7	0	3.7	0.029*

Genetic analysis of the populations showed that repeated genotypes were present at 3 to 15 copies and a majority was repeated only twice. The Fis indices were very low. The low genetic structure observed with microsatellites indicates a high gene flow and/or large effective population sizes.

Conclusions

The observed phylogeny of cytochrome *b* alleles found in European populations of *P. viticola* indicates that QoI resistance alleles belong to two different clades suggesting that resistance appeared independently at least twice in Europe (IR, IIR). The high frequency of QoI resistance in *P. viticola* populations might result from the repeated evolution of resistant alleles in different places. The higher variability in populations of the USA is due to the fact that *P. viticola* exists there since long but was introduced

into Europe only recently (1878). However, given the existence of several sub-species of *P. viticola* in the USA, it is important to elucidate how new species or sub-species develop and to determine whether they present new challenges for disease control (fungicide resistance, cultivar resistance, biological control). In European vineyards haplotype I is more frequently encountered than haplotype II, but haplotype distribution may vary between regions, e.g. in the Champagne vineyard only an average of 10% of haplotype II was observed (Corio-Costet *et al.*, 2006). The biological significance of the two mitochondrial haplotypes is unclear. The combination of neutral and selective genetic markers makes it possible to perform spatio-temporal studies on the spread of sensitive and resistant genotypes and can provide new insights on epidemiological processes. In line with recent results (Gobbin *et al.*, 2006), this study documents a high genotypic diversity (G/N) of *P. viticola* populations which tended to decrease during the season. These results underline the importance of sexual reproduction during winter (oospores as primary inoculum in spring) and an effective asexual multiplication from spring to autumn. The genetic structure of *P. viticola* populations was found to be low indicating a high gene flow and/or large effective population size. For developing models on resistance evolution and implementing sustainable viticulture, it is essential to investigate how resistance levels in treated and untreated *P. viticola* populations develop during the season and from year to year.

Acknowledgements

We thank the colleagues from ITV and CICV for their help in sampling the collection and Aquitaine Region Government for their financial support.

References

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. *Appl. Environ. Microbiol.* **73**, 5162-5172.

Corio-Costet, M-F., Delmotte, F., Martinez, Giresse, X., Raynal, M., Richart-Cervera, S., Douence, L., Panon, ML., Chen, WJ. (2006). Résistance aux QoIs du mildiou de la vigne (*Plasmopara viticola*) : origine et diversité. 8th Int. Conf on Pest and Diseases 2006, pp 612-620, AFPP Eds, CD-Rom.

Delmotte, F., Chen, W-J., Richard-Cervera, S., Greif, C., Papura, D., Giresse, X., Mondor-Genson, G., Corio-Costet, M-F. (2006). Microsatellite loci from the grape downy mildew (*Plasmopara viticola*), *Molecular Ecology Notes* **6** , 379-381.

Gisi, U., Sierotzki, H., Cook, A., McCaffery, A. (2002). Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. *Pest. Manag. Sci.* **58**, 859-867.

Gobbin, D., Rumbou, A., Linde, CC., Gessler, C. (2006). Population genetic structure of *Plasmopara viticola* after 125 years of colonization in European vineyards. *Mol. Plant.Pathol.* **7**, 519-531.

Grasso, V., Palermo, S., Sierotzki, H., Garibaldi, A., Gisi, U. (2006). Cytochrome B gene structure and consequences for resistance to Qo inhibitor fungicides in plant pathogens. *Pest. Manag. Sci.* **62**, 465-472.

Magnien, C., Micoud, A., Glain, M., Remuson, F. (2003). Qoi resistance of downy mildew-monotoring and tests 2002. 7th Int. Conf. on Pest and Diseases 2003, 8 pages. AFPP Eds, CD-Rom.

Raymond, M., Rousset, F. (1995). Genepop (version 1.2), population genetics software for exact tests and ecuminicism. *J. Hered* **86**, 248-249.