Corio-Costet et *al.*, Assessment of Fungicide Resistance in *Erysiphe necator* in French Vineyard Using Quantitative Q-PCR. In: Dehne HW; Deising HB; Fraaije B; Gisi U; Hermann D; Mehl A; Oerke EC; Russell PE; Stammler G; Kuck KH; Lyr H (Eds), "Modern Fungicides and Antifungal Compounds", Vol. VII, pp. 147-149 © 2014 Deutsche Phytomedizinische Gesellschaft, Braunschweig. ISBN: 978-3-941261-13-6

Assessment of Fungicide Resistance in *Erysiphe necator* in French Vineyard Using Quantitative Q-PCR

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INTRODUCTION

Management of the grapevine powdery mildew pathogen *Erysiphe necator* requires numerous treatments with fungicides such as sterol demethylation inhibitors (DMI) and mitochondrial inhibitors (QoI). Recently, reduction in the efficacy of DMI or QoI was reported in Europe and USA (Baudoin et *al.* 2008, Dufour et *al.* 2011). A combined approach based on quantitative PCR (qPCR) of different specific alleles has been developed to detect and quantify the grapevine obligate fungus *Erysiphe necator*, genetically differentiated in two groups (A and B) and to quantify the DMI and QoI resistance (Dufour et *al.* 2011). Here, we present real-time PCR assays using SYBR Green technology developed to detect and quantify sensitivity *vs.* resistance to C14- sterol demethylase inhibitor fungicides (DMI) (Y136F) and quinone outside inhibitors (QoI) (G143A) in vineyards from 2008 to 2012.

EVOLUTION OF ALLELE Y136F, A MARKER INVOLVED IN DMI RESISTANCE FROM 2008 TO 2012

Materials and methods are described in detail in Dufour et *al.* (2011). The relationship between specific allele frequencies and quantification cycle (Cq) for the different assays were linear ($R^2 > 0.97$). The efficacies of qPCR were better than CAPS method with Cq limit of 2 pg for *E. necator* DNA, 0.06 ng for genetic group A and 1.4 ng for DMI-resistance allele. The detection limits of qPCR protocols (LOD) ranged from 0.72 to 0.85% and the quantification limits (LOQ) from 2.4 to 2.85% for the two specific alleles G47A and Y136F, respectively (Dufour et *al.* 2011). The quantitative PCR was then successfully applied to quantify fungal DNA from pooled samples (leaves or bunches) from different French vineyard regions from 2008 to 2012 (Fig. 1 and Fig. 2).

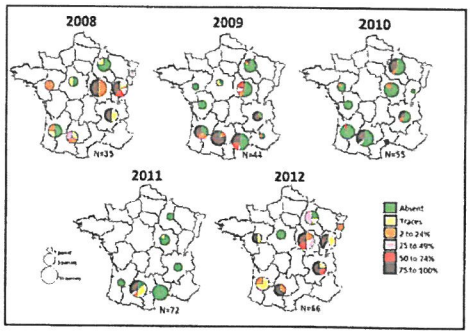

Figure 1 Evolution of allele Y136F (*Cyp 51* gene) in *E. necator* populations in French vineyards 2008 - 2012. Sampling size varied from 35 to 72.

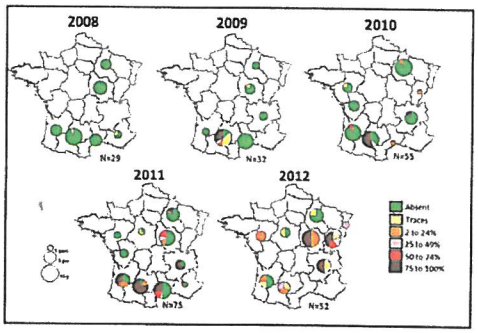

Figure 2 Evolution of allele G143A (cytochrome b gene) in *E. necator* populations in French vineyards 2008 - 2012. Sampling size varied from 29 to 75.

EVOLUTION OF ALLELE G143A, A QOI RESISTANT ALLELE FROM 2008 TO 2012

In addition, the resistance allele (G143A) of QoI was also detected and quantified by q-PCR. The real-time pooled PCR assay differentiated the populations in vineyard without requiring the fastidious isolation of *E. necator* (Baudoin et *al.* 2008, Dufour et *al.* 2011).

CONCLUSION

This work was carried out in the national framework of DMIs and QoIs resistance monitoring. The analyses achieved between 2008 and 2012 showed various situations depending on the administrative areas and on the year for DMI and QoI resistant alleles (Fontaine et *al.* 2012, Fig. 1-3). However, the overall level of allelic frequencies tends to increase over the last two years (Fig. 3).

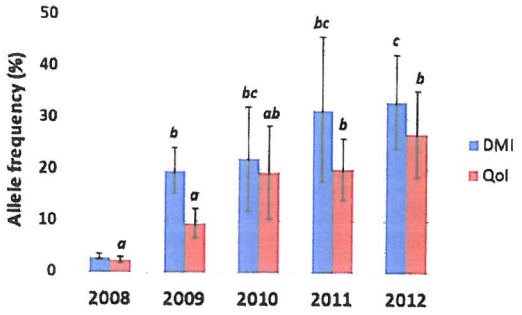

Figure 3 Average of regional frequencies of alleles conferring resistance to QoIs and DMIs between 2008 and 2012.

The real-time PCR assay developed in this study provides a potentially useful tool to efficiently quantify different alleles of interest for fungicide monitoring and for population structure of *E. necator*.

ACKNOWLEDGEMENTS

The authors thank Prof. D Bencené , SRAL, FREDON staff and P. Cartolaro for providing samples and G Taris, Gambier for logistic.

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