American Journal of Plant Sciences, **2012, 3, 1640-1645**
http://dx.doi.org/10.4236/ajps.2012.311199 Published Online November 2012 (http://www.SciRP.org/journal/ajps)

Observation on Baseline Sensitivity of *Erysiphe necator* Genetic Groups to Azoxystrobin

Hajaj Ramadan Hajjeh

Faculty of Agricultural Science and Technology, Palestine Technical University-Kadoori (PTUK), Tulkarm, Palestine.
Email: h.hajjeh@ptuk.edu.ps

Received August 10th, 2012; revised September 17th, 2012; accepted October 15th, 2012

ABSTRACT

Powdery mildew, caused by *Erysiph necator*, is a common and severe fungal disease of grapevine all over the world. The disease costs millions of dollars to vine growers, due to intensive use of fungicides and yield losses. Recently in population of *E. necator* two genetic groups have been described, the two groups seem to occupy different temporal niches, with a temporal alternation that is clear-cut in vineyards intensively treated with chemical fungicides. QoI-STAR (Quinol Outside Inhibitors-Strobilurin Type of Action and Resistance) fungicides are widely used to control the disease, and generally carry a high risk of pathogen resistance development. To clarify the behaviors of the biotrophic fungus when treated with azoxystrobin as a representative of QoI-STAR, baseline sensitivity of laboratory isolates were determined. A leaf bioassay and the primers RSCBF1 and RSCBR2 designed on the highly conserved regions of *cytb* gene in fungi were used. Partial sequence of *E. necator cytb* gene were obtained. Attempts to obtain a laboratory mutant were not totally successful. The sensitivity to azoxystrobin (EC_{50}) in isolates of genetic group B was significantly higher than in isolates of group A, to which all the isolates collected later in the season belonged. The higher sensitivity to azoxystrobin fungicides observed in group B isolates can be at the basis of their precocious disappearance in vineyards, and can have important implications for powdery mildew control strategies.

Keywords: Powdery Mildew; Fungicide Sensitivity; QoI-STAR Fungicide; Genetic Groups; *cytb* Gene

1. Introduction

Powdery mildew, caused by the obligate fungus *Erysiphe necator* Schw., is a common and severe fungal disease of grapevine worldwide, due to the high adaptability of the pathogen to different climatic conditions. In populations of the fungus, two genetic groups or biotypes corresponding to the different overwintering forms have been described (quiescent mycelium in buds, "flag shoot" biotype (group A) and cleistothecia, "ascospore" biotype (group B) [1]. Alternation of the two groups is clear-cut evidence in vineyards intensively treated with chemical fungicides, where group B isolates can persist throughout the season [1]. Thus, changes in the composition of fungal populations might be influenced by spray schedules as a result of different sensitivity to fungicides [2].

Control of the disease is exclusively depending on intensive application of fungicides. Quinol Outside Inhibitors-Strobilurin Type of Action and Resistance (QoI-STAR) fungicides are one of the most important class of agricultural fungicides that are widely used to control grapevine powdery mildew. These fungicides inhibit mitochondrial respiration at the ubiquinol oxidation centre (Qo site) of the cytochrome bc1 enzyme complex (complex III) [3]. Because of their single-site mode of action, QoI fungicides generally carry a high risk of pathogen resistance in more than 30 phytopathogenic species, such as casual agent of powdery mildews, downy mildews, anthracnose, scab, and grey mould [4].

Two mechanisms of resistance to QoI fungicides are known. The first involves one or several point mutations in the *cytb* gene, resulting in changes of the peptide sequence preventing fungicide binding [5]. Single or combined point mutations in the cytochrome *b* gene (*cytb*) were detected in many fungi at amino acid position 127 to 147 and 275 to 296 [6,7]. Substitution of glycine with alanine at position 143 (G143A) was described to be associated with the expression of high resistance, while substitution of glycine to arginine at position 137 (G137R) and substitution of phenylalanine to leucine at position 129 (F129L) are associated with the low resistance [4,8]. The second mechanism was observed *in vitro* and involves's the activity of alternative oxidase (AOX) enzyme, which oxidise ubiquinone and reduce oxygen to water by bypassing the QoI-induced block in the electron transport chain allowing growth [7,9,10].

Field resistance to QoI fungicides in *E. necator* population was detected in USA, several European countries and Australia [11,12]. It has been confirmed that the G143A mutation in *cytb* confers QoI resistance in *E. necator* [13,14].

The development of suitable monitoring techniques and effective anti-resistance strategies are crucial to maintain effectiveness of QoI fungicides. The objective of the present work is to determine the baseline sensitiveity and resistance of *E. necator* genetic groups to azoxystrobin fungicides at molecular level.

2. Materials and Methods

2.1. Fungal Isolates

The fungal isolates used in this study were obtained from the characterized collection at University of Bari [1]. *In vitro* grapevine leaves production and maintenance of the fungal isolates were carried out as described by Miazzi *et al.* (1997) [15].

2.2. Bioassay of Fungicide Sensitivity

Commercial formulations of the QoI fungicides, azoxystrobin (Quadris®, Syngenta), were used. Fungicides were suspended in autoclaved water containing 0.05% tween 20 (Sigma, USA) at final concentrations of 0.1, 0.3, 1, 3, and 6 $\mu g \cdot ml^{-1}$ of active ingredient.

In vitro produced grape leaves of cv. "Baresana" were used to assess the sensitivity of *E. necator* isolates to fungicides [2]. Leaves were standardized in age and size to minimize any possible influence of such factors on the growth of *E. necator* colonies.

Application of fungicides was carried out by dipping leaves in fungicide suspensions at appropriate concentration containing 0.05% tween 20 for 1 min under gentle shaking. Control experiments were conducted by immersing leaves in sterile distilled water containing 0.05% Tween 20. Leaves were then placed in 55-mm-diam Petri dishes containing 10 ml of B0/2 substrate as described by Miazzi *et al.* (1997) [15]. Petri dishes were left closed in a laminar flow cabinet for 8 h before inoculation. Each leaf was then inoculated at single point with about 15 - 20 conidia under a stereomicroscope at 50× magnification. Inoculated leaves were then kept in a growth chamber at 21˚C ± 1˚C and exposed, 16 hours per day, to the light produced by a combination of 3 Osram L36W Cool White lamps and 3 Silvanya Grolux F36W lamps.

After two weeks, diameter of fungal colonies was measured with the aid of stereomicroscope. The effective concentration at 50% (EC_{50}) was calculated for individual isolates. Resistance factor (RF) was calculated according to the formula RF = EC_{50} for the resistant isolate/ EC_{50} of sensitive isolates.

2.3. Molecular Biology Assay

The primers RSCBF1 and RSCBR2, designed by Ishii *et al*. (2001) [16] on the ground of highly conserved regions of *cytb* gene in fungi and proved specific for the *cytb* gene in *Sphaeroteca fusca*, were used.

DNA was extracted from mycelium and conidia with InstaGene Matrix (Bio-Rad Laboratories, USA). PCR was performed in 25 µl reaction mixtures containing 50 ng of total DNA, 2 mM $MgCl_2$, 200 µM each dNTP (Promega, Madison, WI, USA); 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 0.1% gelatin, and 1 U of Red Taq DNA polymerase (Sigma, St Louis, Missouri, USA). PCR reactions were performed in a thermal cycler (Gene Amp PCR System 9700; Perkin-Elmer, Norwalk, USA) programmed as follow: 4 min at 94˚C, followed by 40 cycles of 30 sec at 94˚C, 1 min at 52˚C, 1.5 min at 72˚C, and a final extension stage of 7 min at 72˚C [16]. PCR products were separated on 2% agarose gel (Bio-Rad Laboratories, USA) in 0.5× TBE buffer (45 mM Tris-borate, 1 mM Na-EDTA; pH 8) at 110 V for 120 min (Sub-Cell TM, Bio-Rad Laboratories, USA), The gal was stained with 1 $\mu g \cdot ml^{-1}$ ethidium bromide for 10 min and the bands were recovered by mechanical excision of small gel plugs. DNA fragments were eluted, purified using the Qiaex II Gel Extraction Kit (QIAGEN, Germany) and sequenced by MWG Biotech (Italy).

2.4. Generation of Azoxystrobin Resistant Putative Mutants

Mono-conidial cultures of the G82 isolate, were grown *in vitro* grapevine leaves treated with azoxystrobin. At 1-month intervals, colonies were sub-cultured on leaves treated with increasing concentrations (0.1, 1, 5, 10 and 20 $\mu g \cdot ml^{-1}$) of the fungicide. After that cultures were maintained on leaves treated with 10 or 20 $\mu g \cdot ml^{-1}$ azoxystrobin.

3. Results

Twenty *E. necator* isolates representative of the tow genetic groups (**Table 1**), were used to establish baseline sensitivity to azoxystrobin. EC_{50} values ranged from less than 0.1 to 0.3 $\mu g \cdot ml^{-1}$ azoxystrobin. Colony growth was inhibited at 0.1 to 6 $\mu g \cdot ml^{-1}$ of the fungicide. Differences in EC_{50} values among tested isolates were very low. MIC values showed by isolates belonging to group B were between 1 - 6 $\mu g \cdot ml^{-1}$ azoxystrobin, were slightly higher than that of group A isolates. MIC values of group A isolates wer between 0.1 - 1 $\mu g \cdot ml^{-1}$ azoxystrobin. It is obvious that in most cases, the highest MIC value for group A isolates corresponds to the lowest MIC value for group B isolates.

DNA amplification with the primers RSCBF1 and

Table 1. Sensitivity of *E. necator* isolates belonging to the two genetic group to azoxystrobin.

Genetic group	Isolate	Colonies diameter (mm) of 14-days-old colonies grown on leaves treated with various concentrations (μg·ml^{-1}) of azoxystrobin						EC_{50}	MIC
		0	0.1	0.3	1	3	6		
A "Flag-shoot"	X1	14	10	3	0	0	0	<0.3	1
	X156	7.6	1	2	0	0	0	<0.1	1
	X165	3.3	2	0	0	0	0	<0.3	0.3
	X167		0	0	0	0	0	<0.1	0.1
	X215	5.6	3	1	0	0	0	<0.3	1
	X217	4.3	3	0	0	0	0	<0.3	0.3
	X221	4.5	4.3	1.5	0	0	0	<0.3	1
	X236	4.6	0	0	0	0	0	<0.1	0.1
	X280	4.5	3	3	0	0	0	<1	1
	X283	6.3	5.3	2.5	0	0	0	<0.3	1
	X292	2.6	1.5	0	0	0	0	<0.3	0.3
B "Ascospore"	G82	11	10	5	2	0	0	<0.3	3
	X161	5	2	2	0	0	0	<0.1	1
	X185	7.6	3	2.6	2	0	0	<0.1	3
	X186	7.6	4.3	2	1	0	0	<0.3	3
	X233	5.3	3	1.5	0	0	0	<0.3	1
	X234	5	3.3	2	0	0	0	<0.3	1
	X255	6.3	3	2.6	2.6	2.3	1	<0.1	>6
	X294-A	6.3	1.5	4.3	0	0	0	<1	1
	X296	5.6	4.5	2.3	1	1	0	0.3	6

RSCBR2, of mono-conidial isolate X34, yielded 2 bands of 286 bp (StI) and 224 bp (StII) (**Figure 1**).

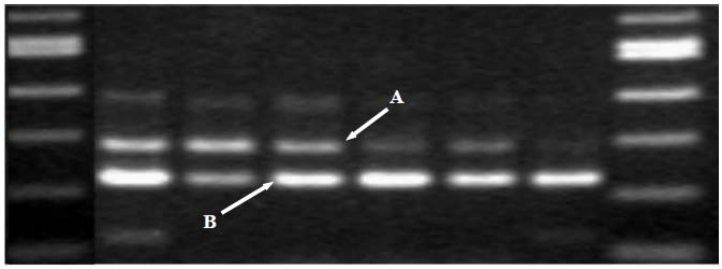
The StI and StII amplicons were sequenced and analyzed with FASTA sequences in Organelles Library of EBI GenBank. Our results showed that StI, but not StII, had a high similarity with the sequences of the mitochondrial *cytb* of other fungi (e.g. *Erysiphe graminis*, *Magnaporthe grisea*, *Venturia inaequalis*, *Podosphaera fusca*, *Saccharomyces cerevisiae*). The similarity within the best 100 scores was 68% - 87.7% identity and 69% - 87.7% for the un-gapped alignment (**Figure 2**).

The StI nucleic acid sequence was translated into amino acid sequence using the ExPASy (Expert Protein Analysis System, Swiss Institute of Bioinformatics, Switzerland; available at website http://us.expasy.org/). The resulting amino acid sequence was aligned with those of other fungi. StI proved to contain amino acid sequence from 53 to 162 of the *cytb* gene, no mutations at G143A and F129L were present (**Figure 2**).

The G82 isolates, were used in experiments for the production of azoxystrobin resistant mutants. Periodical transfer of conidia onto grapevine leaves treated with various concentrations of azoxystrobin resulted in 12 putative mutants of G82 (**Table 2**). The most 6 promising putative resistant mutants (G82-a, G82-d, G82-h, G82-i, G82-j and G82-k) were maintained on leaves treated with azoxystrobin at various concentrations (0, 0.1, 0.3, 1, 5, 10, 20, 100 μg·ml^{-1}). With the exception of G82-i, EC_{50} values for the putative mutants were not distinguishable from that of the parental G82 wild type isolates (<0.1 μg·ml^{-1}). Although MIC values for 5 of them (G82-d and G82-j, MIC > 50 μg·ml^{-1}; G82-h, G82-i and G82-k, MIC = 20 μg·ml^{-1}) were significantly higher than the MIC of G82 (3 - 5 μg·ml^{-1}) (**Table 3**).

4. Discussion

The work herein discussed made a partial sequence of the gene of *E. necator* available; it will be helpful for further



A: St I

```
5'ACAATATCTTGTCCAATTCATGGTATTGCACTCATAAGGTTAGTAATAACTGT
TGCACCTCATAATGACATTTGTCCGTATGGTAAAACGTAACCTAGGAAAGCTGTA
GCCATCATTAATATAAATATTACAACACCTATTGTTCATACTAATGTTCTTGGCG
CTCTGTATGACCCGTTGTATAAACCTCTTCCTATGTGTAAGTAAACTAAAAAGAA
AAAGGCTGAAGCTGTATTTGAGTGTAAGTAACGTATTAATCATCCATTATTTACA
TCTCTCATAATA 3'
```

B: St II

```
5'AACAATATCTTGTCCAATTCATGGACTTTGGCAGGAGTGATAACGATTGTATT
AGCTGGGGCTGGTTTATCATCTTCTTCCCAGTCAATTTCATCTTCTCCTACATGT
TAAATCTTAGCCTGTGTCAATGATTGATTTGCGTCTCTGAGCTAAGATCGACATT
CCTAGAGCTACCTAATTTTCTTTGGGATGTTGGCTTACCATTATTTACATCTCTC
ATAATA 3'
```

Figure 1. Electrophoretic profiles obtained by amplification of *E. necator* DNA with the primers RSCBF1-RSCBR2, and sequences of St I (A) and St II (B).

```
Erysiphe necator (StI)              :FILMMATAFLGYVLPYGQMSLWGATVITNLMSAIPWIGQDIV
Erysiphe graminis (sensitive)       :FILMIVTAFLGYVLPYGHMSHWGATVITNLMSAIPWIGQDIV
Erysiphe graminis (resistant)       :FILMIVTAFLGYVLPYGHMSHWAATVITNLMSAIPWIGQDIV
Magnaporthe grisea                  :LILMMAIGFLGYVLPYGQMSLWGATVITNLISAIPWIGQDIV
Saccharomyces cerevisiae            :FTLTIATAFLGYCCVYGQMSHWGATVITNLFSAIPFVGNDIV
Venturia inaequalis                 :FILMIVTAFLGYVLPYGQMSLWGATVITNLMSAIPWIGQDIV
Podosphaera fusca (sensitive)       :--------FLGYGLPYGQMSLWGATV----------------
Podosphaera fusca (resistant)       :--------FMGYGLPWGQMSLWAATV----------------
                                         *    * **    * ** * ******* ****  * ***
Amino acid position                    :121   129         143             162
```

Figure 2. StI translation nucleic acid to amino acid (underlined) and multiple alignments with the cytochrome *b* gene fragments containing the point mutations responsible of resistance to QoI-STARs in other fungi (in bold, position 129 and 143).

Table 2. Putative mutants of *E. necator* resistant to azoxystrobin obtained by selection of spontaneous mutations.

Starting strain	Colony transfer at 1-month intervals and fungicide concentration (μg·ml^{-1})						Recovered strains
	1st	2nd	3rd	4th	5th	6th	
G82	0.1	1	5				G82-a
			1	5			G82-b
			5	10			G82-c
			1	1			G82-d
			1	10			G82-e
			1	1	10		G82-f
			1	1			G82-g
			1	10			G82-h
			1	1	10	100	G82-i
			1	1	0	20	G82-j
			1	10	20		G82-k
			1	1	10	30	G82-l

Table 3. Putative resistant mutants response to azoxystrobin.

Fungicide concentration ($\mu g \cdot ml^{-1}$)	Diameter (mm) of 14-days colony						
	G82	G82-a	G82-d	G82-h	G82-i	G82-j	G82-k
0	11	12	9	11	4	5	12
0.1	5	5	4	3	3	3	5
0.3	4	4	3	-	3	4	2
1	3	4	2	2	4	2	-
5	0	0	3	-	3	0	2
10	0	0	3	2	4	1	1
20	0	0	0	0	0	0	0
50	0	0	3	0	0	2	0
EC_{50}	<0.1	<0.1	<0.1	<0.1	10 - 20	0.3 - 1	<0.1
MIC	5	5	>50	20	20	>50	20

researches aiming at developing molecular methods useful for field monitoring of the fungal resistance to fungicides.

Attempts of obtaining laboratory mutants resistant to QoI-STAR fungicides were only partially successful, since only few mutants were obtained and usually they displayed a low resistance level. Based on information known in other fungi, mitochondrial resistance may be unstable, since mitochondrial genome is present at a relatively high number of copies, and it is very unlikely that all of them carry resistance mutations [17].

An *in vitro* technique allowed to establish baseline sensitivity of *E. necator* to azoxystrobin fungicide were optimized. No significant differences in response to the fungicides were observed among the tested isolates.

Nevertheless, differences were detected between group A and group B isolates. The same behavior of the two groups was observed in early study in response to triadimenol (DMIs) Fungicide [2].

The higher sensitivity to azoxystrobin of group A isolates might play a role in their disappearance in vineyards from June onwards, when intensive fungicides treatments, including azoxystrobin, are routinely applied. Type A isolates are usually collected on typical flag shoots early in April, at the time of highest susceptibility of shoots to powdery mildew, corresponding to the phenological stage BBCH 13 - 16 (*i.e*. three to six unfolded leaves) [17, 18]. At this time, type A isolates have a higher chance to colonise the forming buds in which they can remain until the next season. This was further confirmed by the fact that the incidence of flag shoots in a vineyard can be predicted by assessing the extent of bud infection in the preceding year [19]. The overwintering mode of group A isolates determines their prevalent asexual reproduction. This seems to be confirmed also by the low genotypic diversity found in this group, and by the unbalanced distribution of the two mating type alleles that characterizes this group [1,20,21]. If isolates of group A are present only at the beginning of the growing season, they have a reduced opportunity to be exposed to the fungicide pressure. This fact, coupled with the absence of the gene rearrangement consequent to sexual reproduction, would make group A isolates less likely to develop resistance.

In contrast, group B isolates develops later in the season and reproduce mostly sexually [22]. Thus, recombinants would have a better opportunity of pyramiding mutations necessary to build the fungicides resistance that these isolates show, and that enables them to play a primary role in epidemics.

Further studies are required for a better understanding of *E. necator* resistance to azoxystrobin and for clarifying the complex interactions that this trait appears to have with population genetics of this pathogen. Ascertaining if the differential sensitivity of isolates of the two genetic groups extends also to other fungicide groups, identifying other factors that may favor one group over the other, and understanding how all these factors can affect the evolution of the epidemics, will be of great help for a more efficient disease control.

5. Acknowledgements

The Author would like to thank Prof. Franco Faretra and Dr. Monica Miazzi from Faculty of Plant Protection and Microbiological Application, Bari University, for their generous support and facilitating carrying out this work.

REFERENCES

[1] M. Miazzi, H. Hajjeh and F. Faretra, "Occurrence and Distribution of Two Distinct Genetic Group in Population of *Erysiphe necator* Schw. In Southern Italy," *Journal of Plant Pathology*, Vol. 90, No. 3, 2008, pp. 563-573.

[2] M. Miazzi and H. R. Hajjeh, "Differential Sensitivity to Triadimenol of *Erysiphe necator* Isolates Belonging to Different Genetic Groups," *Journal of Plant Pathology*, Vol. 93, No. 3, 2011, pp. 729-735.

[3] S. P. Heaney, A. A. Hall, S. A. Davies and G. Olaya, "Resistance to Fungicides in the Qo1-STAR Cross-Resistance Group: Current Perspectives," *Proceedings of Brighton Crop Protection Conference-Pests and Diseases*, Vol. 2, 2000, pp. 755-762.

[4] H. Ishii, "QoI Fungicide Resistance: Current Status and the Problems Associated with DNA-Based Monitoring," In: U. Gisi, I. Chet and M. L. Gullino, Eds., *Recent Developments in Management of Plant Diseases*, *Plant Pathology in the* 21*st Century*, Springer, Dordnecht, 2009, pp. 37-45.

[5] D. Zheng, G. Olaya and W. Koller, "Characterization of Laboratory Mutants of *Venturia inaequalis* Resistant to Strobilurins-Related Fungicides Kresoxim-Methyl," *Current Genetics*, Vol. 38, 2000, pp. 148-155. doi:10.1007/s002940000147

[6] S. Baumler, H. Sierotzki, U. Gisi, V. Mohler, F. G. Felsenstein and G. Schwarz, "Evaluation of *Erysiphe graminis* f. sp. *tritici* Field Isolates for Resistance to Strobilurin Fungicides with Different SNP Detection Systems," *Pest Management Science*, Vol. 59, No. 3, 2003, pp. 310-314. doi:10.1002/ps.639

[7] P. M. Wood and D. W. Hollomon, "A Critical Evaluation of the Role of Alternative Oxidase in the Performance of Strobilurin and Related Fungicides Acting at the Q_o Site of Complex III," *Pest Management Science*, Vol. 59, No. 5, 2003, pp. 499-511. doi:10.1002/ps.655

[8] H. Sierotzki, R. Frey, J. Wullschleger, S. Palermo, S. Karli, J. Godwin and U. Gisi, "Cytochrome b Gene Sequence and Structure of *Pyrenophora teres* and *P. triticirepentis* and Implications for QoI Resistance," *Pest Management Science*, Vol. 63, No. 3, 2007, pp. 225-233. doi:10.1002/ps.1330

[9] M. Miguez, C. Reeve, P. M. Wood and D. W. Hollomon, "Alternative Oxidase Reduces the Sensitivity of *Mycosphaerella graminicola* to QoI Fungicides," *Pest Management Science*, Vol. 60, No. 1, 2004, pp. 3-7. doi:10.1002/ps.837

[10] D. Fernández-Ortuño, J. A. Torés, A. de Vicente and A. Pérez-García, "The QoI Fungicides, the Rise and Fall of a Successful Class of Agricultural Fungicides," In: O. Carisse, Ed., *Fungicides*, Janeza Trdine, Rijeka, 2010, pp. 203-220. doi:10.5772/13205

[11] FRAC, "International FRAC QoI Working Group Minutes 2011, All Crops," Fungicide Resistance Action Committee, 2011.

[12] P. Hoffmann, I. Fuzi and F. Viranyi, "Indirect Effect of Fungicide Treatments on Chasmothecia of *Erysiphe necator* Schwein Overwintering on Grapevine Bark," *Plant Protection Science*, Vol. 48, No. 1, 2012, pp. 21-30.

[13] J. F. Colcol, "Fungicide Sensitivity of *Erysiphe necator* and *Plasmopara viticola* from Virginia and Nearby States," M.Sc. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, 2008.

[14] A. Baudoin, G. Olaya, F. Delmotte, J. F. Colcol and H. Sierotzki, "QoI Resistance of *Plasmopara viticola* and *Erysiphe necator* in the mid-Atlantic United States," 2008. http://www.plantmanagementnetwork.org/pub/php/research/2008/qoi/

[15] M. Miazzi, P. Natale, S. Pollastro and F. Faretra, "Handling of the Biotrophic Pathogen *Uncinula necator* (Schw.) Burr. under Laboratory Conditions and Observations on Its Mating System," *Journal of Plant Pathology*, Vol. 78, 1997, pp. 71-77.

[16] H. Ishii, B. A. Fraaije, T. Sugiyama, K. Noguchi, K. Nishimura, T. Takeda, T. Amano and D. W. Hollomon, "Occurrence and Molecular Characterization of Strobilurin Resistance in Cucumber Powdery Mildew and Downy Mildew," *Phytopathology*, Vol. 91, No. 12, 2001, pp. 1166-1171. doi:10.1094/PHYTO.2001.91.12.1166

[17] A. Rügner, J. Rumbolz, B. Huber, G. Bleyer, U. Gisi, H. Kassemeyer and R. Guggenheim, "Formation of Overwintering Structures of *Uncinula necator* and Colonisation of Grapevine under Field Conditions," *Plant Pathology*, Vol. 51, No. 3, 2002, pp. 322-330. doi:10.1046/j.1365-3059.2002.00694.x

[18] J. Rumbolz and W. D. Gubler, "Susceptibility of Grapevine Buds to Infection by Powdery Mildew *Erysiphe necator*," *Plant Pathology*, Vol. 54, No. 4, 2005, pp. 535-548. doi:10.1111/j.1365-3059.2005.01212.x

[19] M. R. Rademacher, J. Rumbolz and W. D. Gubler, "Evidence for Early Colonisation of Grape Buds by *Uncinula necator*," *Phytopathology*, Vol. 91, 2002, p. S74.

[20] J. P. Peros, T. H. Nguyen, C. Troulet, C. Michel-Romiti and L. Notteghem, "Assessment of Powdery Mildew Resistance of Grape and *Erysiphe necator* Pathogenicity Using a Laboratory Assay," *Vitis*, Vol. 45, 2006, pp. 29-36.

[21] J. Montarry, P. Cartolaro, S. Richard-Cervera and F. Delmotte, "Spatio-Temporal Distribution of *Erysiphe necator* Genetic Groups and Their Relationship with Disease Levels in Vineyards," *European Journal Plant Pathology*, Vol. 123, No. 1, 2009, pp. 61-70. doi:10.1007/s10658-008-9343-9

[22] J. Montarry, P. Cartolaro, F. Delmotte, J. Jolivet and L. Willocquet, "Genetic Structure and s Aggressiveness of *Erysiphe necator* Populations during Grapevine Powdery Mildew Epidemics," *Applied and Environmental Microbiology*, Vol. 78, 2008, pp. 6327-6332. doi:10.1128/AEM.01200-08

