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Proceedings

of the

6th International Workshop

on

Grapevine Downy and Powdery Mildew

GDPM 2010

Edited by :

Agnès CALONNEC
François DELMOTTE
Bob EMMETT
David GADOURY
Cesare GESSLER
Doug GUBLER
Hans-Heinz KASSEMEYER
Peter MAGAREY
Marc RAYNAL
Robert SEEM

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INSTITUT DES SCIENCES
DE LA VIGNE ET DU VIN
BORDEAUX AQUITAINE

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UMR 1065 Santé Végétale INRA -ENITA
Institut des Sciences de la Vigne et du Vin
Centre de recherche Bordeaux-Aquitaine
71, avenue Edouard Bourlaux BP 81
33883 Villenave d'Ornon cedex
France

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6Th International Workshop of grapevine downy and powdery mildew
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Editors

Agnès Calonnec
UMR 1065 Santé Végétale INRA-ENITA, ISVV, 71 Avenue Edouard Bourlaux, 33883 Villenave d'Ornon, France

François Delmotte
UMR 1065 Santé Végétale INRA-ENITA, ISVV, 71 Avenue Edouard Bourlaux, 33883 Villenave d'Ornon, France

Bob Emmett
Department of Primary Industries, PO Box 905, Mildura, Victoria, 3502, Australia

David Gadoury
Cornell University, New York State Agricultural Experiment Station, 14456, Geneva, USA

Cesare Gessler
ETHZ, Universitätstrasse 2, 8092, ETH-Zurich Switzerland, Switzerland

Doug Gubler
Plant Pathology, University of California, 1 Shields Avenue, Davis, CA 95616-8751, USA

Hanns-Heinz Kassemeyer
Department Biology, Staatliches Weinbauinstitut, Merzhauser Stzrasse 119, 79100, Freiburg, Germany

Peter Magarey
Magarey Plant Pathology, PO Box 220, Loxton South Australia, 5333, Loxton, South Australia, Australia

Marc Raynal
Institut Français de la Vigne et du Vin, 39 rue M Montaigne, 33290 Blanquefort, France

Robert Seem
Cornell University, 630 W. North St, 14456-0462, Geneva, NY, USA

Organizers:
Agnès Calonnec and François Delmotte, UMR 1065 Santé Végétale, INRA-ENITA, 71 av. Ed. Bourlaux, 33883 Villenave d'Ornon, France
Marc Raynal, Institut Français de la Vigne et du Vin, 39 rue M Montaigne, 33290 Blanquefort, France

Local organization
Jean-Marc Armand, Inra
Philippe Cartolaro, Inra
Laurent Delière, Inra
Alain Girard, Inra
Marie Lauwerier, Inra
Marie-Christine Médalin, Inra
Pierre Sauris, Inra

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Preface

We are pleased to welcome you to this **6th International Workshop on Grapevine Downy and Powdery Mildew**. For the first time since its creation in Geneva in 1991, the conference is being hosted in Bordeaux, one of the oldest and most famous viticulture regions in France. We are very proud to have been able to convene 98 people from 15 different nations and five continents, which demonstrate the importance and geographic extent of grape and its mildews and the shared interest of the international community to develop a sustainable viticulture. This meeting will take place both in Bordeaux city center and at the new Institut de la Vigne et du Vin (ISVV) located at Villenave d'Ornon. These ideal locations will give you the opportunity to discover the fantastic metamorphosis of a city worthy of its rank as a UNESCO World Heritage Site and also to meet the people involved in research, academic teaching and technology transfer related to viticulture and oenology.

National and international government regulations are increasingly restrictive regarding the use of phytochemical treatments. Thus, the development of viticulture and related policies significantly increase the need to improve our ability to control grapevine diseases with respect to the environment while maintaining yield and 'typicité'. Therefore, the challenge ahead for grape mildew research requires the exchange of hypotheses and findings from a variety of scientific disciplines such as plant pathology and epidemiology but also population genetics and ecology, molecular plant-pathogen interaction, plant physiology, grapevine breeding and genomics. Next step towards a sustainable grape protection will call for the integration of these multi-scales approaches. Until 2002, the workshop was mainly dedicated to epidemiology and disease forecasting, but in its 5th edition in Italy (2006) – under the impetus of I. Pertot and C. Gessler – the workshop has begun to attract a broader audience such as from the disciplines stated above. For this 6th edition, following the lead of the inter-disciplinary approach taken by the Italian meeting, we have encouraged all researchers interested in downy and powdery mildews of grapevine research to participate. As a result of this, we present a diverse and interesting program of sessions.

These proceedings compile 50 oral presentations and 26 posters that will be presented during the five day long meeting, provided by four main sessions that are dedicated to either the host, the pathogen or the disease. These are: (1) Breeding, induced resistance, plant-pathogen interaction, (2) Biology of the pathogen, ontogenic resistance, pathogen impact on plant physiology, population genetics, (3) Detection methods, monitoring, epidemiological modelling, disease management, (4) Disease management, disease economic impact, desision model, forecasting models, fungicide efficacy, biocontrol. We believe the four different but complementary sessions provide a representive overview of powdery and downy mildew research today and we hope they will permit to reach our goal by stimulating inter-disciplinary discussions.

We would like to take this opportunity to thank our colleagues in UMR 1065 Plant Health (Inra Bordeaux) that have provided invaluable help in making this meeting in Bordeaux possible. In particular, we would like to mention Jean-Marc Armand, Philippe Cartolaro, Laurent Deliere, Marie Lauwerier, Marie-Christine Médalin, Pierre Sauris and also Alain Girard from the Inra press office. We also would like to thank Bayer CropScience SAS, the Institute de la Vigne et du Vin (ISVV), the Conseil Regional d'Aquitaine (CRA), the Conseil Interprofessionnel du Vin de Bordeaux (CIVB) and the Société Française de Phytopathologie (SFP) and Inra, for their generous contributions for funding this meeting. We would also like to note that the workshop has also received strong support from the grapevine growing community including small growers to the different 'Syndicats des Appellations' and the 'grands Chateaux' of Bordeaux. They have opened their doors, some of them will receive you on our day trip and they all provided us with the wines that you will have the chance to enjoy during the meeting.

We welcome you to this 6th edition of the workshop and we hope that the program we have devised will allow the exchange of ideas and collaboration as well as the chance to make new friends and to initiate new projects for the future!

Agnès Calonnec
François Delmotte
Marc Raynal

Sponsors

We gratefully acknowledge the generous grant support provided by the following agencies, corporate and industrial sponsors:

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UMR GDPP,IBVM-INRA Virologie Végétale INRA Bordeaux
Avenue Edouard Bourlaux, BP 81 33883 Villenave d'Ornon
Cedex - France

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Conseil Interprofessionnel du Vin de Bordeaux
1, cours du XXX Juillet - 33075 Bordeaux Cedex - France

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ODG de Bordeaux et Bordeaux Supérieur
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PROGRAMM OF THE MEETING

Sunday 4 July

18h - 21h	**Distribution of meeting material and welcome party at the faculty of Pharmacy**	

Monday 5 July

	Faculty of Pharmacy	Presenting author
8h30 - 9h	***Distribution of meeting material***	
9h-9h30	**Introduction to the meeting**	
9h30-10h30	**S1. Breeding, host resistance**	
	Genetic analysis of the resistance to downy and powdery mildews derived from cultivar Bronner	Paule Blasi
	Development of a phenotyping platform for assessment of resistance to grape downy and powdery mildews	Sabine Merdinoglu
	Physiological and biochemical analysis of responses of Tunisian grapevine varieties to powdery mildew disease (*Uncinula necator*)	Ahmed Mliki
10h30-11h	***coffee break***	
11h-12h30	Development of resources for comparative physical mapping between *Muscadinia Rotundifolia* and *Vitis vinifera*	Zah-Bi CI
	Genetic Diversity and Mechanism of Host Resistance to Downy Mildew in Oriental *Vitis* Species	Jiang Lu
	New selection tools for resistance breeding	Pierre-Henri Dubuis
	Functional and molecular characterization of grapevine resistance induced by *Trichoderma hartianum* T39 against *Plasmopara viticola*	Michele Perazzolli
12h30-14h	***lunch***	
14h-15h00	**poster S1**	
15h00-16h00	**S1. Induced resistance, plant-pathogen interaction**	
	Comparaison of phosphonate derivatives (fosétyl-Al, PK2) efficacy to that of BTH as grapevine defence elicitors against *Plasmopara viticola*	Marie-Cécile Dufour
	Side effects of the herbicide glufosinate ammonium on *Plasmopara viticola* and other fungal pathogens	Andreas Kortekamp
	Towards the identification of avirulence genes from *Plasmopara viticola*, the causal agent of grapevine downy mildew	Pere Mestre
16h00-16h30	***coffee break***	
16h30-17h40	Cytological and molecular analyses of the first infection steps of *Erysiphe necator* reveal its interaction with the host plant	Hans-Heinz Kassemeyer
	Characterization of the necrosis producing protein NPPPV from *Plasmopara viticola* belonging to the Nep1-like protein family (NLPs) and its putative role in the host-pathogen-interaction	Hans-Heinz Kassemeyer
	Sporulation in *Erysiphe necator*: Signals, differential gene expression and practical implications for disease management	Robert Seem
	15 ' for conclusions about S1	
18h-20h	**Visit of the Historic Bordeaux city centre**	

Tuesday 6 July

	Faculty of Pharmacy	Presenting author
9h00-12h15	**S2. Biology of the pathogens, ontogenic resistance, pathogen impact on plant physiology, Population genetics**	
	Acute Low Temperature Events Reduce the Survival of *Erysiphe necator* and Increase Resistance in Ordinarily-Susceptible *Vitis vinifera* Leaf Tissue	Michelle M. Moyer
	Effect of Prior Vegetative Growth, Inoculum Density and Light on Conidiation in *Erysiphe necator*	David Gadoury
	Dynamic of ontogenic resistance and growth variation in the interaction powdery mildew-grapevine	Sylvain Schnee
	Maximum severity of powdery mildew on grape leaves coincides with the sink to source transition	Katherine Evans
10h20-10h50	***coffee break***	
	Can early population structure of Erysiphe necator inform about the disease level on bunches?	François Delmotte
	Photosynthetic activity in grape leaf tissue with latent, visible and 'virtual' downy mildew lesions	Tito Caffi
	Downy mildew control based on the plant physiology	Jermini Mauro
	Invasion history of grapevine downy mildew (Plasmopara viticola) :a population perspective	François Delmotte
	15 ' for conclusions about S2	
12h15-14h	***lunch***	
14h-15h20	**poster S1-S2**	
15h20-16h20	**S3. Epidemiology: detection methods, monitoring, epidemiological modeling**	
	Aerobiology of *Erysiphe necator* in northern viticulture	Odile Carisse
	A multiplex polymerase chain reaction assay for the detection and identification of *Plasmopara viticola, Erysiphe necator* and *Botrytis cinerea* spores in airborne environmental samples	Vanesa Huerga
	Classical conditioning of domestic honeybees to olfactory stimuli associated with grapevine powdery mildew infections	Doug Gubler
16h20-16h50	***coffee break***	
16h50-17h50	Downy and powdery mildew spore monitoring in Rioja Alavesa vineyards	Ana Diez
	Effect of the grapevine growth on the dynamics of a powdery mildew epidemic: field trials and simulations	Agnès Calonnec
	Modelling the life cycle of *Erysiphe necator*	Elisabetha Legler
18h45-20h	***Cocktail at the Bordeaux City Hall with presentation of the Bordeaux vineyards and wine tasting***	

Wednesday 7 July

Day trip

8h-10h15	***Visit of the INRA experimental station of Latresne***
11h	***Visit of the Cave Coopérative "Rauzan" in the Entre-deux-Mers region***
13h	***Lunch at "Rauzan"***
14h30	***Visit of a "Grand Château" in Saint-Emilion***
16h	***Visit of the the monolithic church***
19h-23h00	***Intronisations & Dinner at Saint-Emilion***

Thursday 8 July

	ISVV	Presenting author
8h00-9h	***Transportation from Bordeaux to Villenave d'Ornon***	
9h-9h30	***Presentation of the ISVV institut***	
9h30-10h10	**S3. Epidemiological knowledge and Disease management**	
	Toward establishing low input regimes in Australian viticulture 2: Observations on the spatial movement of downy mildew, *Plasmopara viticola* , after a single secondary infection event in an South Australian vineyard	Peter Magarey
	Can cultivar mixtures in organic vine growing reduce downy mildew severity?	Cesare Gessler
10h10-10h30	***coffee break***	
10h30-12h10	Early symptoms assessment as indicator to control Grapevine Powdery mildew epidemics with an optimal number of fungicide applications	Philippe Cartolaro
	Toward establishing low input regimes in Australian viticulture 3: Use of 'epi-season' and 'lag phase control' in applying epidemiological knowledge, reducing the number of sprays and inoculum reservoirs for long-term control of grapevine powdery mildew	Peter Magarey
	Effect of sunlight, specifically ultraviolet radiation and increases in surface temperature, on grapevine powdery mildew development	Craig N. Austin
	Toward establishing low input regimes in Australian viticulture 1: A review of powdery mildew control in vineyards of the Riverland, South Australia	Peter Magarey
	Trials results of the Optidose method using an adjustment of the pesticide dose for control of downy and powdery mildew	Alexandre Davy
	15 ' for conclusions about S3	
12h25-12h45	***presentation of the INRA experimental station and wine from Château Couhins***	
12h45-14h	***lunch***	
14h-14h45	**poster S3-S4**	
14h45-16h25	**S4. Economic impact, Forecasting models, decision models**	
	Integrated grapevine powdery and downy mildew management in south eastern Australia: Evaluation of the impact of long term research and development	Bob Emmett
	Global economic importance of Grape Powdery and Downy Mildew protection	Dominique Steiger
	Testing a decision system for Integrated Protection against Mildews: the vine-grower, the adviser, and the computer model	Olivier Naud
	A Bio-Economic Model to Evaluate and Compare Different Protection Strategies Against Grapevine Downy and Powdery Mildew	Pascal Leroy
	EPIcure, a geographic information decision support system risk assessment of downy and powdery mildew epidemics in Bordeaux vineyards	Marc Raynal
16h25-16h50	***coffee break***	
16h50-17h50	McLaren Vale CropWatch: A Case study of the practical application of Downy Mildew modeling for the McLaren Vale grape growing region of South Australia.	Jodie Armstrong
	Downy and powdery mildew models integtrated in the forecasting system "VitiMeteo"	Gotfried Bleyer
	The expert system OiDiag-2.2	Walter K. Kast
18h-18h30	***Transportation from Villenave d'Ornon to Bordeaux***	
20h-23h30	***Gala dinner at the place de la Bourse***	

Friday 9 July

ISVV

8h00-9h	***Transportation from Bordeaux to Villenave d'Ornon***	Presenting author
9h-12h00	**S4. Fungicides efficacy, Biological control**	
	Continuous research to propose solutions against powdery mildew	Gilbert Labourdette
	Ecology, Toxicity and Efficacy; How fungicides for downy mildew and powdery mildew in grapes have changed since 1970.	Hugh D Armstrong
	Application of Profiler fungicide (a.i. fosetyl-Al+ fluopicolide) in control of grapevine downy mildew in Montenegro	Nedeljko Latinovic
	Comparison of fosetyl-Al and another phosphonate on plant Downy mildew protection and on Arabidopsis thaliana Gene Expression	Pascale Latorse
10h20-10h50	***coffee break***	
	Production and eradication of overwintering inoculum of *Erysiphe necator* in Michigan vineyards	Annemiek Shilder
	Control of foliar diseases in viticulture using milk: understanding mechanisms	Dale Godfrey
	Is the biocontrol efficacy of *Ampelomyces quisqualis* against powdery mildew related to the aggressiveness of the strain?	Dario Angeli
	Alternatives to copper for controlling grapevine downy mildew in organic viticulture	Ilaria Pertot
12h00-12h45	***Wine tasting***	
12h45-14h00	***lunch***	
14h-15h30	**poster S3-S4** + *coffee break*	
15h30-16h30	**Conclusions**	
17h	***Return to Bordeaux***	

Table of contents

Session 1: Breeding, induced resistance, Plant-pathogen interactions

Genetic analysis of the resistance to downy and powdery mildews derived from cultivar Bronner

P. Blasi[a], S. Schnee[b], S. Wiedemann-Merdinoglu[a], E. Prado[a], S. Godard[b], P. Coste[a], C. Onimus[a], K. Gindro[b], C. Schneider[a], O. Viret[b], D. Merdinoglu[a]

[a]INRA-UDS, UMR 1131 Santé de la Vigne et Qualité du Vin, 28 rue de Herrlisheim, BP 20507, 68021 Colmar cedex, France ; [b]Département Fédéral de l'Economie DFE, Station de recherche Agroscope Changins-Wädenswil ACW, CP 1012, 1260 Nyon, Suisse

A wide range of pathogens threatens viticulture. Among them, downy and powdery mildews are the most important in Europe. The current strategy to control these diseases relies totally on the use of fungicides. This practice is not only expensive but also causes a slow and progressive damage to the environment. A cost-effective and environment friendly alternative to the use of chemicals is the development of varieties resistant to pathogens. All traditional European grapevine varieties are susceptible to the main pathogens. However *Vitis* species closely related to cultivated grapevine were shown to be potential sources of resistance to a wide spectrum of grapevine diseases (Boubals 1959, Staudt and Kassemeyer 1995).

The absence of private grapevine breeders in France led the INRA to design a breeding program dedicated to create new resistant varieties. The main goal of this programme is to create varieties durably resistant to downy and powdery mildews with a berry quality suitable to produce high quality wines (Merdinoglu *et al.* 2009). In order to successfully reach the double objective of high resistance efficiency and durability, the use of multiple sources of resistance was planned as soon as the project was designed. The project was developped in close interconnection with upstream research programmes which aim at understanding the genetic bases of the resistance to downy mildew derived from grapevine-related wild species by addressing four key questions: (i) exploring the diversity available in genetic resources to chose original genitors (ii) identifying and characterizing the relevant genes/QTLs to genetically improve the targeted traits, (iii) using the data acquired on genes/QTLs (position, effects) to assist the selection with markers, and (iv) assessing the durability of the identified resistance genes/QTLs.

In the presented study, we analysed the genetic determinism of the resistance to grapevine downy and powdery mildews derived from cultivar Bronner. We used two BC6 mapping populations, respectively consisting of 96 and 143 individuals from a cross between the resistant parent Bronner and an other resistant parent derived from *Muscadinia rotundifolia* var. Dearing. The two populations were screened with two SSR markers flanking Rpv1, a downy mildew resistance QTL from *Muscadinia*, in order to discard the individuals with this resistance factor and only keep the plants segregating for the resistance derived from Bronner. Resistance to downy mildew was assessed after artificial inoculation on both populations. Plants were genotyped at 65 SSR loci which allowed us to build a genetic map covering 648 cM. The interval mapping analysis revealed the presence of a major downy mildew resistance QTL that was located on linkage group 9. This resistance factor accounted for 52% of the total phenotypic variation. Thus, we considered this QTL as a major gene.

Resistance to powdery mildew was assessed after artificial inoculation as well. The interval mapping analysis revealed the presence of two minor QTLs involved in powdery mildew resistance. The two QTLs were located on linkage group 3 and linkage group 19 and explained 8% and 16% of the total phenotypic variation, respectively.

Literature cited

Boubals D. 1959. Contribution à l'étude des causes de la résistance des Vitacées au mildiou de la vigne et de leur mode de transmission héréditaire. *Ann. Amelior. Plant.* 9 :1-236.

Merdinoglu D, Merdinoglu-Wiedemann S, Mestre P, Prado E, Schneider C. 2009. Apport de l'innovation variétale dans la réduction des intrants phytosanitaires au vignoble : exemple de la résistance au mildiou et à l'oïdium. *Progrès Agricole et Viticole* 126:244-247.

Staudt G, Kassemeyer HH. 1995. Evaluation of downy mildew resistance in various accessions of wild *Vitis* species. *Vitis* 34:225-228.

Development of a phenotyping platform for assessment of resistance to grape downy and powdery mildews

S. Wiedemann-Merdinoglu, V. Dumas, M.A. Dorne, E. Duchene, P. Mestre, D. Merdinoglu

INRA-Colmar, UMR INRA-Université de Strasbourg 1131, Laboratory of Plant Breeding, 28 rue de Herrlisheim BP 507. 68021 Colmar, France

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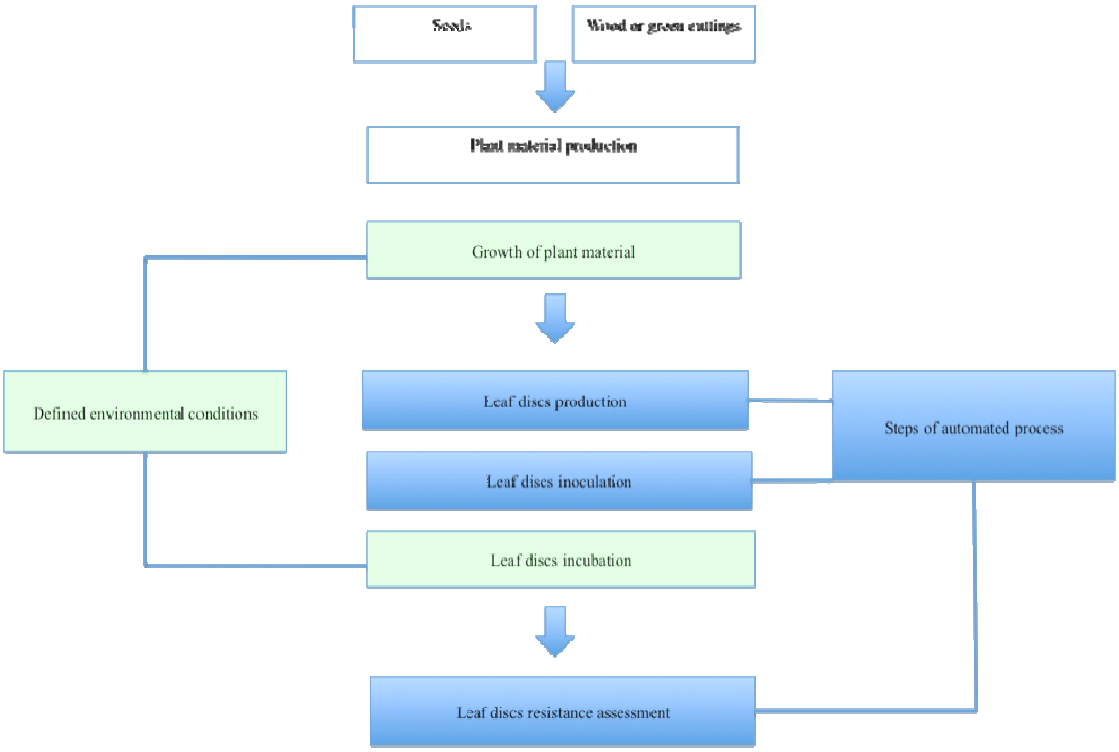
Breeding for downy and powdery mildew resistant varieties is an alternative to the intensive use of pesticides which have a negative effect on environment and on human health.
After the identification of some resistant sources among wild *Vitis* species, their genetic determinism are unraveled by associating genotypic information with corresponding phenotyping data. If progress in DNA markers enable high throughput genotyping of large plant populations, phenotyping for resistance is still laborious. Field and greenhouse screening using resistance scale are widely used by breeders. However, this approach is time consuming and may be affected by environmental conditions which have an inpact on disease progression.

To overcome this weak point, a phenotyping platform for resistance assessment has been developed. This tool is based on a laboratory leaf disc bioassay and is suitable for the following skills: 1) to provide large plant populations grown in homogeneous and controlled conditions, 2) to manage simultaneous inoculations with different strains of a pathogen, 3) to improve the throughput, accuracy and reliability of resistance assessment, 4) to reduce the time and the space needed to achieve the phenotyping process. For this purpose, several steps of the phenotyping process were standardized or automated by the acquisition or the development of new facilities such as specific climate chambers, robots and image analysis system.
The phenotyping platform, from plant material production to resistance assessment, will be presented.



Physiological and biochemical analysis of responses of Tunisian grapevine varieties to powdery mildew disease (*Uncinula necator*)

N. Zghonda, A. Mliki, S. Chebil

Laboratoire de Physiologie Moléculaire des Plantes, Centre de Biotechnologie de Borj Cédria, BP 901, Hammam-Lif 2050, Tunisia (*ahmed.mliki@cbbc.rnrt.tn)

The sensitivity of 50 Tunisian grapevine cultivars (*Vitis vinifera* L.) to the powdery mildew fungus, *Uncinula necator,* has been studied. The results revealed a differences in the behaviour of those cultivars to the pathogen as certain varieties such as Ferrani demonstrated a tolerance to the disease whereas others like Razegui were susceptible. In fact, with the autochthonous variety "Ferrani", the symptoms appeared 7 days later than with the susceptible variety Razegui.
The analysis using two-dimensional electrophoresis of the total proteins, extracted from leaves deriving from inoculated and control plants of these two contrasting varieties, revealed that certain proteins were induced and others inhibited. Moreover, the mineral analysis of dry material from control and inoculated leaves showed an increase of K^+, Cl^- and Fe^{2++} ions concomitantly with a decrease of Ca^{2+}.

Susceptibility of the autochthonous varieties to the infection with of *Uncinula necator*

The fungal attack is expressed as the % of the leaf surface occupied by the mycelium (average of 10 leaves). The severity of the attack was annotated from 0 to 5 where 0 means no infection, 1:0-5%, 2:5-25%, 3:25-50, 4:50-75 and 5:>75% of leaf surface (Table 1).
According to the results presented in Table 1, after two weeks pos-inoculation of the fungus, only variety Ferrani displayed a surface of attack less than 5% (score: 1). However, after three weeks, the fungus spread on all developed leaves from all of the varieties including Ferrani.

Proteins induced following the inoculation of *Uncinula necator*

Total proteins were extracted from leaves of the varieties ferrani (as relatively tolerant) and Razegui as susceptible. For both varieties, the total proteins decreased one week post-inoculation and slightly increased after two weeks (Table 2).

Table 1: Scores of the odium disease for the different tunisian grapevine varieties two weeks post-inoculation of *Uncinula necator*.

Variety	Dgrees of the infection
Dallia	3
Asli	3
Beldi Rafraf	4
Farrani	1
Ahmer bou Ahmer	3
Hencha 1	3
Chaouch	3
Balta 1	4
Arbi Abiadh	3
Sakasly	2
Dabbouki	2
Hencha H2	2
Blanc 3	3
Khamri Tozeur	3
Marsaoui	3
Châaraoui Rafraf	4
Hamri Kerkennah	2
Muscat Rafraf	3
Bidh Hamem Sfax	4
Khedhiri 3	3
Akhel Meguergueb	2
Sfaxi S2 Nafta	2
Arbia	2
Razegui	4
Djebbi	3
Saouadi	3
Bahbahi	4
Guelb Sardouk	3
Meguergueb Djerba	4
Turky	3
El biodh	4
Arich Djerba	4
BKB Gabes	4
Arich Dressé	4
BKB Sfax	4
Bidh Hamem Rafraf	3
Djerbi Dguech	4
Arich Ahmer	4
Beldi Baddar	3
Bezzoul Khadem Rafraf	4
Khamri	4
Tounsi Djerba	3
Khedhiri 1	3
Khedhiri 2	2
Blanc 1	2
Beldi Sayeb INRST	2
Medina	2
Mahdaoui	2
Balta 2	3
Kahli Sfax + kahli kerkennah	3

Table 2: Total proteins from Ferrani and Razegui

Sample	Total proteins (mg/g of fresh leaves)
Variety Razegui	
Control, after 1 week	3,21
Inoculated, after 1 week	2,23
Control, after 2 weeks	1,66
Inoculated, after 2 weeks	1,72
Variety Ferrani	
Control, after 1 week	3,58
Inoculated, after 1 week	1,67
Control, after 2 weeks	2,52
Inoculated, after 2 weeks	2,59

Two dimensional electrophoresis of total proteins from Ferrani and Razegui

Extracts were analyzed by 2D-electrophoresis and gels were stained with silver nitrate. The results revealed that confirmed the increase of the total proteins two weeks post-inoculation in both varieties.

The figure 1 shows that, the inoculation of *Uncinula necator* resulted in the induction and the inhibition of the biosynthesis of certain proteins. However, the induced or inhibited proteins are not the same for both varieties. This would explain the difference of the morphological response (degrees of susceptibility) of these varieties to the fungus.

Effect of the infection on the mineral composition of the leaves

Globally, we noted a change in the mineral composition (Ca^{2+}, Cl^{-}, K^{+}, Fe^{2+}) of the leaves from the infected plants of both varieties Ferrani and Razegui. However, the results were not able to discriminate clearly between the two varieties in terms of susceptibility and tolerance.

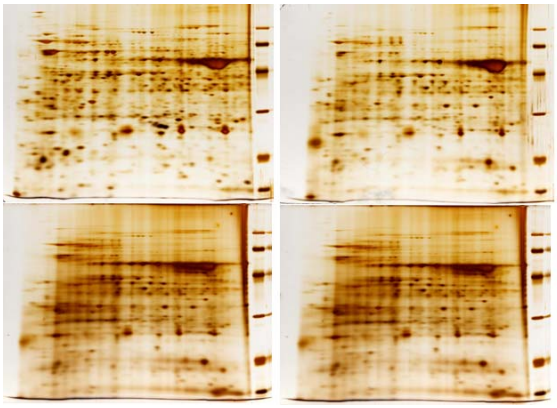


Figure 1: 2D-PAGE of total proteins extracted two weeks post-inoculation. Upper: Ferrani (left: control, right: inoculated). Lower: Razegui (left: control, right: inoculated)

Conclusion

As expected, all of the inoculated grapevines were susceptible to the inoculation of *Uncinula necator*. However, the mycelium development on certain varieties such as Ferrani was slower and less intense. Furthermore, the inoculation resulted in the induction and inhibition of sets of proteins which were different from one variety to another.

Development of resources for comparative physical mapping between *Muscadinia rotundifolia* and *Vitis vinifera*

C. I. Zah-Bi[a], S. Blanc[b], M. Bras[c], A. Canaguier[a], I. Le Clainche[a], L. Couturat[d], N. Choisne[c], I. Dry[e], M. Gouyvenoux[d], D. Merdinoglu[b], J. Poulain[d], H. Quesneville[c], P. Wincker[d], P. Mestre[b], A.F. Adam-Blondon[a]

[a] UMR-INRA-UEVE-CNRS Génomique Végétale, 2 rue Gaston Crémieux, BP5708, 91 057 Evry cedex, France; [b] UMR INRA-US Santé de la Vigne et Qualité du Vin, 28 rue de Herrlisheim, 68021 Colmar, France ; [c] Unité de Recherches Génomique-Info, route de Saint-Cyr 78026 Versailles, France ; [d] Genoscope, CEA-IG, 2 rue Gaston Crémieux, BP 5708, 91057 Evry cedex, France ; [e] CSIRO Plant Industry, PO Box 350, Glen Osmond SA 5064, Australia.

A cost-effective and environment friendly alternative to the use of chemicals is the use of varieties resistant to pathogens. However, for *Vitis vinifera* L. (2n=38), the cultivated grapevine, the resistance needs to be introduced from other Vitaceae through breeding programs ensuring wine quality. Among them, *Muscadinia rotundifolia* (2n=40) is closely related to the *Vitis* genus and is a source of efficient resistance to several pathogens used as a genitor in breeding programs at INRA. However, despite its importance for grapevine breeding, our knowledge about genetics/genomics of *M. rotundifolia* is very limited. Comparative mapping in both species would speed up the identification and isolation the different resistance genes from *M. rotundifolia* and a better understanding of the mechanisms associated to the introgression of genome segments from *M. rotundifolia* in *V. vinifera*. For this purpose, two resources are under development in *M. rotundifolia* cv Regale: a genetic map in a full sib progeny and a Bacterial Artificial Chromosome (BAC) library for physical mapping.

Material and methods

A BAC library of *Muscadinia rotundifolia* cv Regale was constructed according to a protocol modified from Adam-Blondon *et al.* (2005) using *Hind*III and *Bam*HI digested nuclear DNA. The average size of insert was estimated for each sub-library, according to Adam-Blondon *et al.* (2005). The BAC-end sequences (BES) were obtained as described in Lamoureux *et al.* (2006). The percentage of inserts corresponding to chloroplastic DNA was estimated through *in silico* analysis of the BES: when the two BES of a clone were aligned on the grapevine chloroplast sequence, the insert was counted as derived from chloroplastic DNA. The parameters for the alignment of the BES on the *Vitis vinifera* genome were the following: the two BES from a single clone had to show a unique match of 500bp length minimum to the reference genome sequence, the two matches have to be on the same chromosome and their distance is above 20kb or below 150kb.

Results

Four sub-libraries were obtained, 3 using the *Hind*III digested DNA and 1 using the *Bam*HI digested DNA and stored in one hundred and twelve 384 plates. The BAC library thus consists of 54,174 clones. The characteristics of each sub-library are given in table 1. The average size of inserts was rather low compared to previous libraries (Adam-Blondon *et al.*, 2005): 59 to 82kb. The percentage of empty clones was quite high (6% to 14% depending of the sub-library) whereas the chloroplastic contamination was comparable to the one observed for other grapevine libraries by Adam-Blondon et al 2005. Taking into account all these parameters, this BAC library may represent 7X the *M. rotundifolia* genome, giving a 91.49% probability of identifying a clone corresponding to any *Muscadinia rotundifolia* DNA sequence.

Table 1: Characteristics of the *Muscadinia rotundifolia* cv Regale. The number of clones does not take into account the empty clones.

Library (CNS name)	Enzyme	Average size of the inserts	Empty clones (%)	Chloro-plastic clones (%)	Clone number
AEMOAAA	*Hind* III	75 kb	6.27	3.1	15774
AEMOAAB	*Hind* III	82 kb	6.81	2.4	10368
AEMOAAC	*Bam*HI	59 kb	13.97	2.4	13440
AEMOAAD	*Hind* III	73 kb	9	2.4	14592

BAC library**.** A total of 86,810 BES were obtained and aligned on the *V. vinifera* reference genome sequence as a starting point for physical comparative mapping (Figure 1). Thirteen thousand and thirty-two BES of the 86810 BES have showed a unique match to the reference genome sequence and the two BES from a clone have been on the same chromosome.

The ongoing work is now focusing on two regions, one on chromosome 12 and one on chromosome 18 where QTL for resistance to powdery or downy mildew have been detected and containing clusters of NBS-LRR (Moroldo *et al.*, 2008). *Run1*, a single dominant gene present in *M. rotundifolia*, has been introgressed into *V. vinifera* and genetic and physical mapping allowed to construct a BAC contig (made from an introgressed individual) between the SSR markers VMC4f3.1 and VMC8g9 on chromosome 12 (Barker *et al.*, 2005; Figure 2). This contig of BACs still contains a gap and correspond to a region with a cluster of NBS-LRR encoding genes (Donald *et al.*, 2002, Barker *et al.*, 2005 and Dry *et al.*, 2010). The SSR markers VMC4F3-1, VMC8G9 and UDV-058 could be aligned on the *V. vinifera* genome sequence which was not the case for any of the BAC end sequence-derived markers developed by Barker et al (2005) CB46.49, CB13.14 and 49MRP1.P2. This shows that, as expected, the microsynteny is not very good in regions containing clusters of NBS-LRR.

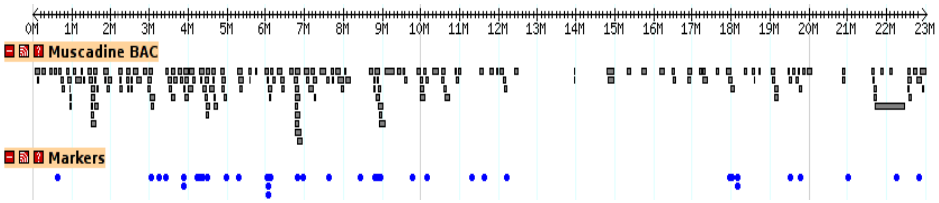


Figure 1. Snapshot of a view on the genome browser showing the alignment of the *M. rotundifolia* BACs on chromosome 1 of the grapevine reference genome.

In parallel, a *M. rotundifolia* genetic map is under development, using SSR markers already mapped in *V. vinifera* (Doligez *et al.* 2006; Cipriani *et al.* in prep). A total of 351 SSR markers have been tested for amplification on Regale and 12 S1 individuals, 144 yielding successful amplification and heterozygosity. The markers are currently scored on 175 individuals of a Regale S1 population.

Perspectives

A complete contig of BAC from the *M. Rotundifolia* library will be constructed by PCR screening of the library with markers developed by Barler *et al.* (2005) and new markers developed from BES. The BAC will be sequenced. The same approach will be carried out on chromosome 18 along a QTL of resistance to downy mildew (Bellin *et al.*, 2009).

Finally, the alignment of the *M. rotundifolia* genetic map to the grapevine reference genome will enlight the macrosynteny between the two genomes.

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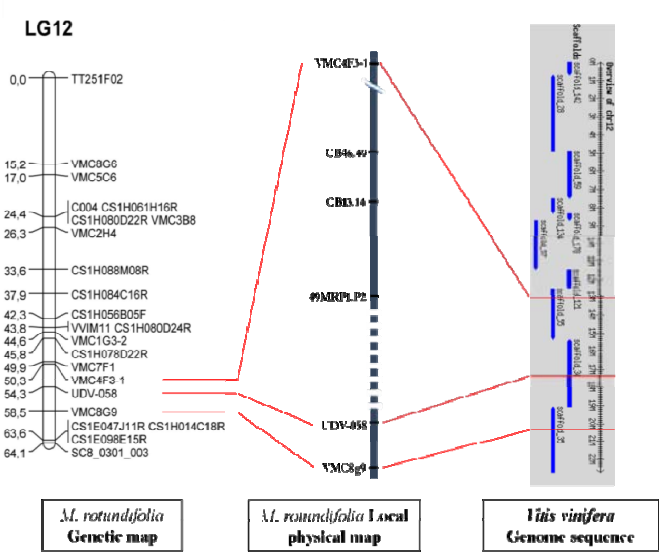

Figure 2. Alignment of the *V. vinifera* reference genetic map (Cipriani et al in prep), the physical map of an introgressed individual carrying the Run1 gene (Barker et al 2005) and the *V. vinifera* reference genome sequence.

Genetic Variation and Mechanism of Host Resistance to Downy Mildew Disease among Oriental *Vitis* Species

J. Lu[ab], Y. Zhang[a,b], J. Wang[b]

[a]Center for Viticulture and Small Fruit Research, Florida A&M University, Tallahassee, Florida, USD; [b]Center for Viticulture and Enology, China Agricultural University, Beijing, China

It has been well known that most North American native grapes are resistant to *Plasmopara viticola* (PV), the pathogen causing grape downy mildew (DM) disease, while European grapes are susceptible to the DM disease. However, the resistance and genetic variation are less certain among the oriental grapes species. This study is therefore designed to evaluate the DM resistance among the major Chinese *Vitis* species, and to understand the mechanism of host resistance in morphological, tissue, cellular and molecular level of these grape species. Among them, *V. quinquangularis* and *V. pseudoreticulata* are found to be the two most PV resistant oriental *Vitis* species. *V. quiquangularis* is originated and distributed in South China where a sizable viticulture industry has been developed from *V. quiquangularis* clonal selections and hybrids. *Vitis amurensis*, the most cold-hardy grape species, is resistant to PV in general but intraspecific variations are noticeable among different accessions evaluated. Variations of DM resistance are also observed among the interspecifc hybrids between the oriental species and *V. vinifera* grapes. Based on our observation, the genetic variation and DM resistant level of these oriental grapes are compatible to the North American grape species.

In order to understand the mechanism of DM resistance among the oriental grapes, microscopy study was used to analyze the host resistance in tissue and cellular level. In the mean time, to identify genes and pathways that are involved in resistance to grape DM disease, a gene-expression based molecular analysis was conducted in *V. amurensis* cv. Zuoshan-1, a DM resistant cultivar. Solexa sequencing technology was used for estimating gene expression level by "deep sequencing" transcripts derived from PV infected and non-infected leaves. A near complete set of transcripts (up to 8 million 21 bp tags) derived from control (CON) and PV infected (INF) cDNA libraries were sequenced. Comparative analysis between the CON and INF libraries revealed a large number of pathogen-induced up- and down-regulated genes, and a good number of them were "highly" differentially expressed as much as 50 folds or more between the CON and INF libraries. Real Time RT-PCR was used to further confirm these differentially expressed transcripts and their involvement in responding to the PV infection. Pathway enrichment analysis was used to identify significantly enriched pathways from these differentially expressed genes.

New selection tools for resistance breeding

K. Gindro[a], J.-L. Spring[a], S. Godard[a], P.-H. Dubuis[a]

[a]Agroscope Changins-Wädenswil ACW, Route de Duillier, CH-1260 Nyon

Worldwide the vast majority of the grapevine areas is planted with *Vitis vinifera* cultivars that are all highly sensitive to both downy and powdery mildew. The consequence is that multiple fungicide applications are needed to secure the production of high quality wines. Since no alternative product can reach the level of efficacy of chemical fungicides, new high quality resistant cultivars are a promising way to reduce the negative impacts of these chemicals. Since 1996, Agroscope Changins-Wädenswil runs a breeding program for resistant grapevine cultivars. The selection objectives are: high downy mildew resistance, low sensitivity to powdery mildew and grey mold, good agronomical characteristics, and high oenological potential. In order to reduce the time needed to select resistant cultivars a new method (Gindro *et al.*, 2006, 2007 a), based on histological and biochemical criteria, has been developed to evaluate quickly and accurately the level of seedlings' resistance to downy mildew (*Plasmopara viticola*). Twenty four hours after artificial inoculation (hpi) of seedlings with downy mildew the level of callose-like material accumulation is evaluated by fluorescence microscopy (Gindro *et al.* 2003). Synthesis of ε-viniferin and δ-viniferin, two specific stilbenes, are analysed by chromatography at 48 hpi. Five days after inoculation the sporangia density is assessed either with a spectrophotometer or evaluated visually. This approach allows a rapid selection based on objective criteria. Only the most resistant plants are selected for further agronomical and oenological evaluation. To date 3500 seedlings were tested and 169 (4.8%) were resistant and were further analysed for their agronomical and oenological potential.

Furthermore, the efficacy of various fungicides or elicitors (i.e. activators of plant defense mechanisms) and their action on the pathogen and the plant were evaluated using the same resistance markers (Gindro *et al.*, 2007 b). Detached leaves as well as whole plants of *Vitis vinifera* cv. Chasselas where used for evaluation with the resistance criteria (rate of infection, quantification of sporulation, quantification of stilbenic phytoalexins). To date, 40 products, issued either from the industry, or from organic and biodynamic production, were tested.

The results show that only very few products have a satisfactory efficacy in these *in-vitro* and greenhouse tests. For example two plant extracts, *Rheum palmatum* root extract and *Frangula alnus* bark extract are able to protect leaves from *Plasmopara viticola* (Godard *et al.*, 2009). They are toxic to the downy mildew and induce plant defense mechanisms in *Vitis vinifera* cv. Chasselas. Other products lead to a very good protection efficacy against downy mildew, due to important fungitoxic effects without induction of plant defense mechanisms. It is important to mention that all tests with alternative products were done *in-vitro* and in greenhouse. That is why the actual efficacy in field conditions of these products must be tested thoroughly before any conclusion on their efficacy can be drawn. The described method allows a reliable evaluation of the elicitation potential of plant protection products against downy mildew of grapevine based on objective analytical parameters.

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Functional and molecular characterization of grapevine resistance induced by *Trichoderma harzianum* T39 against *Plasmopara viticola*

M. Perazzolli[a], B. Roatti[a], O. Giovannini[a], I. Pertot[a]

[a] IASMA Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all'Adige, TN, 38010, Italy, E-mail: *michele.perazzolli@iasma.it*

Downy mildew, caused by the biotrophic oomycete *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni, is one of the most destructive grapevine diseases, and its control is based on the repeated application of chemical fungicides. For health and environmental reasons, there is increasing governmental and public pressure to reduce reliance on pesticides for disease control in viticulture and focus on alternatives to chemicals, such as biological control agents (BCAs). *Trichoderma* spp. are cosmopolitan fungi, widely used against several plant pathogens. They act by different mechanisms, such as competition, antibiosis, parasitism or activation of plant resistance (Shoresh *et al.*, 2010). Enhancement of plant resistance is a frequent reaction to some beneficial microorganism, and it is referred to induced systemic resistance (ISR; Pieterse *et al.*, 2009). In contrast to systemic acquired resistance (SAR), ISR is usually characterized by a broad-spectrum resistance and associated to a priming state. Primed plants display faster or stronger activation of defense responses after pathogen infection, without major fitness costs under pathogen-free conditions (Van Hulten *et al.*, 2006). Thus, ISR can be a promising strategy for controlling crop diseases. In grapevine, increased resistance against important pathogens can be induced by non-pathogenic microorganisms or elicitors, but limited information is available on the mechanisms of resistance activation. Our aim is to investigate the metabolic costs and molecular processes involved in resistance against downy mildew, induced by *T. harzianum* T39 in susceptible grapevines.

Material and methods

Plants of the susceptible *Vitis vinifera* cv. Pinot noir were grown under greenhouse controlled conditions. Water suspensions of Trichodex (Makhteshim Ltd., Israel), corresponding to 10^5 conidia/ml of *T. harzianum* T39, and of 0.5 g/l of BTH (Bion; Syngenta) were used as resistance inducers. The 4-5 basal leaves or 5-6 leaves on one side of each shoot were treated three times with the resistance inducers 1, 2 and 3 days before pathogen inoculation (Perazzolli *et al.*, 20008). As control treatment, plants were sprayed with water (untreated control) or copper hydroxide (Kocide 2000, Du Pont de Nemours).

For pathogen inoculation, *P. viticola* sporangia suspension was sprayed to abaxial leaf surfaces and plants were incubated overnight at 95% RH. Disease severity was assessed 10 days after inoculation as percentage of infected leaf area (sporulation) of treated (local effect) and untreated (systemic effect) leaves.

For the analysis of metabolic costs, plants were treated three times per week with water or with the resistance inducers. Plant growth was evaluated by counting the number of leaves on each plant, by measuring shoots, leaves and roots fresh and dry weight. Chlorophyll content of all leaves was weakly evaluated as leaf transmittance measured by a SPADmeter (Steele *et al.*, 2008).

For gene expression analyses, RNA was extracted before and 24 h after pathogen inoculation from treated and untreated leaves of ISR-expressing and control plants. The cDNA was obtained and expression of grapevine markers of salicylate or ethylene/jasmonate pathways (Perazzolli *et al.*, 2010) was analyzed by real-time RT-PCR.

Results and discussion

Leaf treatments with *T. harzianum* T39 increased grapevine resistance against downy mildew both locally and systemically (Figure1). On treated leaves *T. harzianum* T39 reduces the disease severity similarly to the standard copper treatment. On untreated leaves, resistance activation was comparable after treatment of basal leaves or treatment of leaves on one side of shoots, suggesting a similar acropetal and lateral systemic activation of the resistance. The absence of a direct effect of *T. harzianum* T39 on *P. viticola* sporangia and the systemic disease reduction on untreated leaves indicated that a plant-mediated defense mechanism was the main processes involved the *T. harzianum* T39-induced resistance (Perazzolli *et al.*, 2008).

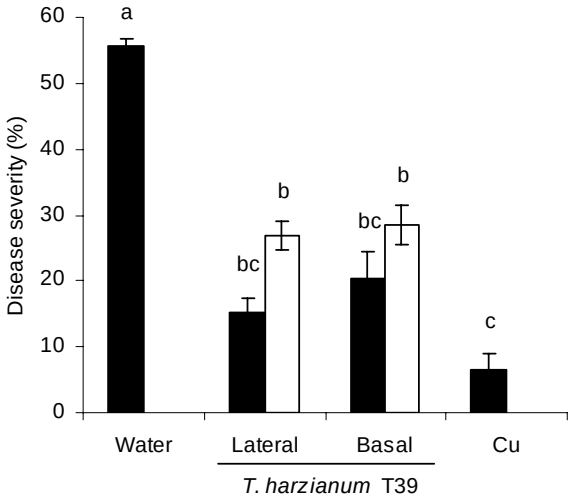

Figure 1: Effect of treatments with *Trichoderma harzianum* T39 on downy mildew severity. Basal leaves (Basal) or the leaves of one side of each shoot (Lateral) were treated with the biocontrol agent. Downy mildew severity was evaluated locally (black) and systemically (white) and compared with Water- or copper-treated (Cu) plants. The mean severity and standard error of nine replicates for each treatment are reported. Different letters indicate significant differences (Tukey's test, $\alpha = 0.05$).

In absence of pathogen infection, repeated *T. harzianum* T39 treatments did not reduce plant growth, measured as number of leaves (Figure 2) or fresh and dry weight of leaves, shoots and roots. In contrast, applications of

benzothiadiazole (BTH), which is a SAR inducer, strongly affected grapevine growth starting from the 5th week of treatments. The chlorophyll content was comparable in leaves of *T. harzianum* T39-treated and untreated control plants, and it was significantly reduced in BTH-treated leaves.

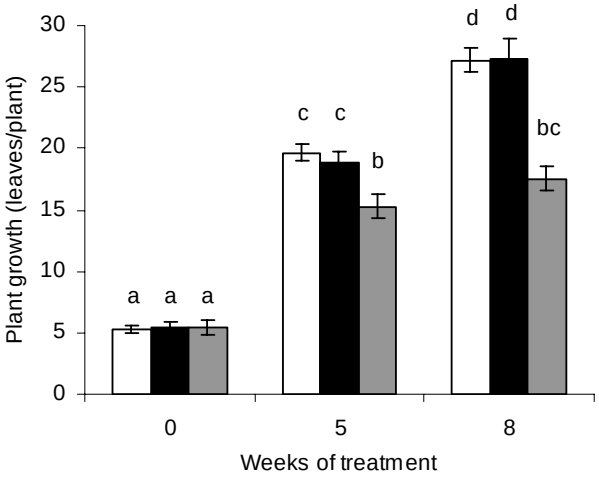

Figure 2: Effect of repeated water (white), *Trichoderma harzianum* T39 (black) or benzothiadiazole (grey) treatments on grapevine growth, measured as number of leaves/plant. For each treatment the mean severity and standard error of ten replicates are reported; different letters indicate significant differences (Tukey's test, α = 0.05).

The absence of evident metabolic costs in *T. harzianum* T39-treated plants suggested the activation of a priming state in grapevine. However, gene expression analyses revealed that grapevine markers of defense signaling pathways (Perazzolli *et al.*, 2010) were not primed for augmented up-regulation after *P. viticola* infection. A genome-wide gene expression analysis will help in the identification of the mechanisms and key genes involved in the grapevine resistance induced by *T. harzianum* T39. More knowledge of the ISR activation processes is necessary in order to optimize the *T. harzianum* T39-induced self-protection, which at the moment did not display a sufficient disease control for an application under commercial field conditions.

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Comparison of phosphonate derivatives (fosétyl-Al, PK2) efficacy to that of BTH as grapevine defence elicitors against *Plasmopara viticola*

M. C. Dufour[a] and M. F. Corio-Costet[a]

[a] INRA, Bordeaux, UMR Santé Végétale (1065) , BP, 81, 33883 Villenave d'Ornon, France.

Since the introduction of the causal agent of grapevine downy mildew (*Plasmopara viticola*) from America in 1878, the control of outbreaks requires numerous chemical treatments having consequences on the environment and the health. One of the means of reducing the use of chemicals is to modify agricultural practices. It is thus urgent to develop and to support the sustainable agriculture that it is integrated and\or agro-biological. So stimulating defences, in addition to resistant varieties and biological warfare, is an interesting method of alternative pest management.
Because they act on the plant and not directly on the pathogenic, elicitors lead to multifactorial resistance of the plant host which means that pathogen should find it more difficult to get round.

The UMR Santé Végétale unit at INRA Bordeaux ha developed studies to work out new strategies integrating the use of additional methods (biological, plant selection, or elicitors) in pest management control. The use of plant defence stimulators, apart from seeing reasoned use of plant treatment products, seems promising.

It is well known that phosphonates have a powerful antifungal activity and the fungicide fosetyl-Al (*O*-ethyl phosphonate) (1, 2) is known to exert both a direct effect on the pathogen and an indirect effect via stimulation of host defences. In this present study, the efficiency of two phosphonate derivatives, Fosetyl-Al and a foliar fertilizer (PK2) (3) was compared to Benzothiadiazol (BTH) (4), a salicylic acid analogue, against *Plasmopara viticola* (5).

The assessement of efficacy was made at several levels, biological (efficacy on pathogen growth), biochemical (quantification of phenolic compounds) and molecular (gene expressions). The aim was to understand better how these elicitors work, and also to provide some answers with regard to the interest of developing alternative strategies for induction of plant defence during the growing season in vineyard. Trials in the vineyard, on experimental plots treated with the various compounds, were carried out to estimate the efficacy of elicitation methods under natural conditions.

Material and Methods

Leaf disks of Cabernet Sauvignon pretreated with a range of concentration of PK2, Fosetyl and BTH, and inoculated with 4 different strains of downy mildew, were used to check the potential ED_{50} of elicitors. Grapevine defence efficacy assessment were carried out on leaf disks and controls were carried out by sprinkling sterile distilled water. Each elicitor was applied at 6 dilutions on eight disks by pulverization with the various concentrations from 0 to 7 mM. Three independent experiments were performed with downy mildew strains.
Treatment efficacy was determined by visual assessment of percentage of growth of the pathogen on the leave disks after 7 days at 22°C. The results were presented as an average percentage of inhibition compared of fungus growth compared to control, using the following calculation:

$$\% \text{ growth inhibition} = 100 \times \left(\frac{\%''\text{treated}'' \text{ growth}}{\%\ ''\text{control}'' \text{ growth}} - 1 \right)$$

By means of graphic representations of the percentage of inhibition according to compound concentrations, a value of Effective Dose 50 (ED50) corresponding to the dose inhibiting in 50 % the growth was determined.

Secondly, leaves were pre-treated with the various compounds 24 hours before pathogen inoculation and gene expression levels of 20 genes known to play a part in plant defences mechanisms were quantified by RT-PCR for three days after the pathogen inoculation. We followed the expression of genes coding for enzymes of phenylpropanoïd biosynthesis (PAL, STS, CHS, CHI, LDOX and BAN), phytohormone biosynthesis (JA, SA and ethylene) LOX, ACC PAL and genes coding for PR proteins (CHIT4c, PGIP, PIN, GLU, PR1 and PR10).
RNA was isolated from frozen tissues samples by phenol-chloroform extraction followed by lithium chloride precipitation according to the method of Reid and al. (2008). The extraction buffer contained 300 mM Tris HCl (pH 8.0), 25 mM EDTA, 2 M NaCl, 2% CTAB, 2% PVPP, 0.05% spermidine trihydrochloride, and just prior to use, 2% β-mercaptoethanol. Tissue was ground to a fine powder in liquid nitrogen and was added to pre-warmed (65°C) extraction buffer at 20 ml/g of tissue. Tubes were subsequently incubated in a 65°C water bath for 10 min and shaken. Mixtures were extracted twice with equal volumes chloroform:isoamyl alcohol (24:1) then centrifuged at 3,500 × g for 15 min at 4°C. To the supernatant, 0.1 vol 3 M NaOAc (pH 5.2) and 0.6 vol isopropanol were added, mixed, and then stored at -80°C for 30 min. Nucleic acid pellets were collected by centrifugation at 3,500 × g for 30 min at 4°C and dissolved in 1 ml Tris-EDTA Buffer (pH 7.5). After a selective precipitation of RNA, 0.3 vol of 8 M LiCl was added and the sample was stored overnight at 4°C. RNA was pelleted by centrifugation at 20,000 × g for 30 min at 4°C then washed with ice cold 70% EtOH, air dried, and dissolved in DEPC-treated water. RNAs were quantified after DNase I digestion by absorbance measure at 260 nm and 280 nm and 260/280 nm ratios were determined (Genequant pro, Amersham Bioscience, France). cDNAs were synthesized by reverse-transcription using 2 µM oligo $d(T)_{15}$ and using Promega reagents. The reverse transcription reaction mix is assembled on ice to contain nuclease-free water, M-MLV reverse transcriptase (Promega) and its 5X reaction buffer, dNTPs, and ribonuclease inhibitor according to the manufacturer's instructions. An initial annealing at 94°C for 5 min, the

reaction is incubated at 42°C for 1hr. The obtained cDNA was stocked at -20°C.

Several common housekeeping genes were selected for expression analysis (Table 1).

Real-time PCR reactions were determined using 96-well plates with an iCycler thermocycler (Bio-Rad france, Ivry sur Seine) using SYBR® Green. For each reaction, 1µl of each primer at 10nM, and 7 µl of 2X- Blue SYBR Green fluorescein Mix including Hot start DNA polymerase, dNTP and $MgCl_2$ (Abgene, France) and 5µl of cDNAs, were used in compliance with manufacturer's instruction. Amplifications were measured under the following conditions: a first step at 94°C for 15 min, and 40 cycles at 94°C for 10 s, 55°C for 10 s, and 72°C for 20 s. Relative gene expression was obtained with the formula: fold induction = $2^{-[\Delta\Delta Ct]}$, where ΔΔCt = [Ct GI (unknown sample) - Ct EF1γ (unknown sample)] - [Ct GI (reference sample) - Ct EF1γ (reference sample)]. GI is the gene of interest, and EF1γ is the grapevine Elongation Factor1 γ gene used as the internal control. The reference sample is the sample chosen to represent 1 fold expression of the gene of interest (e.g., control leaves untreated and no inoculated). Each sample was assayed in duplicate at the minimum.

Table 1. Primer sets used for the grapevine defence genes expression.

Gènes	N° accession GeneBank
Chaine gamma du facteur d'élongation 1 **(EF1γ)**	AF176496
Phénylalanine ammonia lyase **(PAL)**	X75967
Stilbène synthase **(STS)**	X76892
PR protéines de classe 1 **(PR1)**	AJ536326
PR protéines de classe 10 **(PR10)**	AJ291705
Chalcone isomérase **(CHI)**	X75963
Chalcone synthase **(CHS)**	X75969
Chitinase de classe III **(CHIT3)**	Z68123
Chitinase de classe IV **(CHIT4)**	VVU97521
Protéine inhibant les polygalacturonases **(PGIP)**	AF305093
β 1,3 glucanase **(GLU)**	AF239617
Inhibiteur de sérine protéase **(PIN)**	AY156047
Lipoxygénase **(LOX)**	AY159556
Leucoanthocyanidine dioxygénase **(LDOX)**	X75966
Glutathione S- transférase **(GST)**	AY156048
Anthocyanidine réductase **(BAN)**	VVI000166
Acide 1-aminocyclopropane, 1-carboxylique synthase **(ACC)**	AF424611
Antranilate Synthase **(ANTS)**	XM 002281597
Chorismate Mutase **(CHORM)**	FJ604854
Chorismate Synthase **(CHORS)**	FJ604855

Experimental field tests were carried out on an experimental plot of land, established by the repetition of 4 blocks consisting of three vine stocks of Cabernet Sauvignon, treated weekly with 2 g /L of BTH, 2.5 g/L de fosetyl and 2,1 g/ L of Dithane (reference fungicide). Treatments began on May 5th, 2009 at the stage 13-14 of the BBCH scale (3-4 leaves unfolded) and stopped on July 21st at the stage 79 of the BBCH scale (berries reach their final size), namely 12 treatments. Untreated control blocks were also introduced into the experimental plot. An artificial inoculation of *P. viticola* was carried out on May 27th, (stage 55, BBCH), 24 hours after the fourth treatment, at the rate of 6 leaves by vine stock pulverized with a solution of 45 000 sporangia per ml.

The development of the disease was then measured every week by estimating the percentage of leaves attacked and the severity on leaves.

Results:

Laboratory assays showed that BTH, Fosetyl and PK2 were uniformly effective against *P. Viticola* (Figure 1).

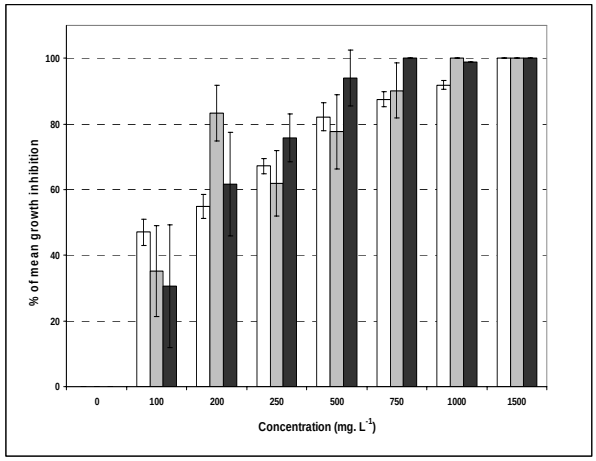

Fig.1. Effect of BTH (white bars), FOS (grey bars) and PK2 black bars) on *Plasmopara viticola* growth.

Leaf disks were inoculated 1 hour after being sprayed with different concentrations of BTH (white bars), FOS (grey bars) and PK2 (black bars). Data were the mean of 2 independent experiments.

We obtained no significant differences in efficacy (P = 0.917) between the various compounds.

BTH, Fosetyl-Al and PK2 all showed good efficacy against downy mildew and allowed to 100 % of inhibition (figure 1). The values of ED_{50} were 0.47 mM, 0.50 mM and 0.96 mM, respectively. However no significant difference existed between the various groups (data not shown).

Concerning the level of gene expression, no significant difference between the control and the treated with the various products in the absence of pathogens was detected. A significant repression of the expression of the majority of the genes was observed after inoculation with strains of pathogens alone ($P < 0.05$, data not shown). Significant differences were also found in leaves pretreated with the various elicitors and in the presence of the pathogen. In the presence of BTH with downy mildew, PR proteins were specifically overexpressed (PR1, GLU, LOX and CHIT3) (Table 2).

Table 2. Balance of significant overexpressed genes depending on different treatments.

	PK 2			FOS			BTH		
	24 hpi	*48 hpi*	*72 hpi*	*24 hpi*	*48 hpi*	*72 hpi*	*24 hpi*	*48 hpi*	*72 hpi*
CHI	**27,0**	4,2	2,0	1,7	9,8	1,6	0,4	6,3	1,6
CHS	1,2	1,6	3,2	1,1	2,8	**13,4**	0,5	2,2	2,3
LDOX	0,1	5,8	1,5	0,1	3,5	0,4	**0,0**	2,1	3,5
PR1	7,4	10,9	2,5	1,8	6,4	**22,2**	4,6	**93,1**	2,6
PR10	**14,0**	4,4	1,8	**6,5**	8,9	2,6	6,2	9,2	2,0
CHIT3	0,7	1,8	0,6	2,0	3,2	2,3	4,6	**9,7**	0,2
CHIT4	**33,3**	4,1	5,5	**14,4**	4,7	3,5	2,6	8,9	5,4
PGIP	**26,1**	0,6	**3,3**	16,2	10,4	3,9	0,2	3,6	0,9
GLU	**0,1**	1,0	1,1	7,7	**3,2**	2,3	0,7	**5,7**	0,1
PIN	4,4	**8,0**	**7,4**	0,5	7,1	4,5	2,5	13,9	3,4
LOX	1,5	0,7	3,1	2,1	1,9	0,8	0,6	**2,9**	0,0

Concerning phosphonates, they essentially led the over expression of genes coding for the anthocyanin biosynthesis

(CHI, CHS) and for PR proteins (PR1, PR10, PGIP, GLU and CHIT4) in the presence of *P. viticola* (Table 2).

The field trial carried out in 2009 revealed that the outbreak began its development from June 18th with a new phase taking place from July 9th until August 20th when more than 90 % of leaves control plots were attacked (figure 2A and 2B). On grapes, more than 90 % severity was reached in July. No grapes remained at the date of September 1st in control plots.

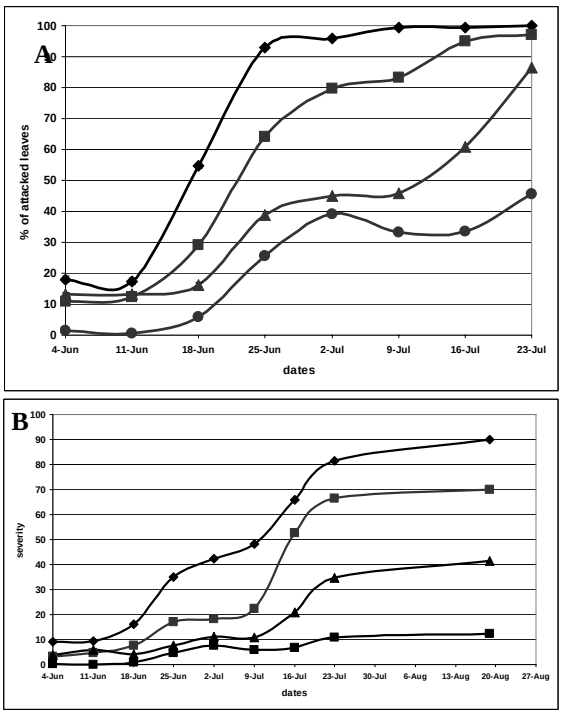

Fig. 2. Evolution of downy mildew outbreak in 2009 for the 4 product tested (BTH (square), FOS (triangle), Dithane (circle) and Control (diamond)). **(A)** % of attacked leaves, **(B)** severity (% of mean attack).

Under experimental conditions, the reference fungicide, showed good efficacy throughout the experiment, on both leaves and grapes.

Fosetyl also presented a good efficacy until July 16th and showed an attack on leaves of only 40 % at August 20th in comparison with the control where it was more than 90 %. For grapes, 20 % of grapes were affected in June, and in July the mean percentage of grape attack reached 70 %. By September 1st, some grapes remained but were very affected. BTH also provided good protection until July 9th, but leaves were then very quickly affected and severity reached 70 %. On the contrary, for grapes in June severity was only 20 %, as with Fosetyl, but on the other hand, in July better protection was conferred by BTH than with Fosetyl. Indeed BTH lead to 40 % of severity on grapes against 70 % with Fosetyl. It should be noted, however, that the vines treated with BTH still had fine, undamaged grapes in the date of September 1st, but with delayed ripening.

Conclusion:

This study showed that the answers of the plant after various elicitor treatments lead to efficacies and to modulation of gene expression depending on the product used. So, BTH, a salicylic analogue, favours the over expression of PR proteins such as PR1, chitinase 3 and Glucanase. On the contrary, phosphonates lead to over expression of the anthocyanin biosynthesis pathway, and also of PR proteins such as PR10, Chitinase 4, Polygalacturonase in the presence of *P. viticola.* All in all the downy mildew leads to gene overexpression involved in anthocyanin biosynthesis s and PR proteins.

The methods developed in our study to assess the stimulating effect and the efficacy of elicitors enabled us to obtain a better understanding about the real potential of elicitors against downy mildew. To complete the assays, biochemical analysis of phenylpropanoids is in progress. The possibility of following the outbreaks and gene expression in vineyard conditions also enlightened us as to the real state of grapevine defence after elicitation and before or after attacks of the pathogen. The field experiment carried out in 2009 will be carried out again in 2010. This will allow us to compare the real efficacy of products on downy mildew. The biochemical and molecular analyses of leaves taken at different times will be of interest to understand and assess the possibility of using elicitors as an alternative or additional method in pest management of the grapevine.

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Side effects of the herbicide glufosinate ammonium on *Plasmopara viticola* and other fungal pathogens

A. Kortekamp

DLR Research Station, Breitenweg71, D-67435 Neustadt an der Weinstrasse, Germany.

The downy mildew pathogen *Plasmopara viticola* passes through a series of developmental stages during infection and colonization of the host that have been well studied cytologically (e.g., Kortekamp *et al.* 1998; Kortekamp 2005). In compatible interactions, the pathogen is guided by host signals to stomata (Kiefer *et al.*, 2002) representing the primary ports of entry, grows from the cell that was initially invaded and proliferates intercellularely for several days until sporulation occurs. The specific signals that induce sporulation are unknown, but the process is influenced by environmental conditions such as humidity and light (Rumbolz *et al.* 2002). Due to its high multiplication rate, the downy mildew pathogen is probably the most notorious of all grape pathogens, hence being responsible for severe crops losses each year, and *P. viticola* is classified as a pathogen showing a high risk of development of resistance to fungicides (Russel, 2004). Furthermore, the risk of residual fungicides in the environment or the final product has spurred the interest in alternative means of managing downy mildew diseases. This refers especially to organic agriculture, where copper-based fungicides are still used to combat downy mildews.

Additional tools to manage downy mildew disease include the resistance pool present in American and Asian *Vitis* species, and breeding efforts led to the introduction of new tolerant varieties. Even though some molecular aspects of the host-parasite interaction have already been studied (Kortekamp, 2006, Kortekamp *et al.*, 2008, Polesani *et al.*, 2008), the molecular mechanisms of resistance are still far from being well known.

A cDNA-AFLP analysis of plant and pathogen genes expressed in grapevine leaves of a susceptible variety infected with *P. viticola* revealed that the majority of *P. viticola* transcripts expressed *in planta* has a homology to genes of unknown function or to genomic *Phytophthora* sequences (Polesani *et al.,* 2008). However, genes related to metabolism, energy production, transport and signal transduction were also identified. Many grapevine genes were down regulated during late infection time points, especially those involved in photosynthesis, but there are confusing results regarding protein metabolism. Some genes involved in protein biosynthesis seemed to be induced, whereas others displayed a repressed expression. One interesting gene differentially expressed by the host was a cytosolic glutamine synthetase, which seems to be induced during the initiation of an infection but repressed at later time points when infected leaf areas are close to become senescent. Glutamine synthetase (GS) catalyses the first reaction in the main pathway of ammonia assimilation in higher plants leading to a set of amino acids after transformation of glutamine.

Interestingly, elevated expression of GS occurred in germinating cysts of *Phytophthora nicotianae* (Shan *et al.*, 2004), representing the stage at which plant colonization is initiated. Grenville-Briggs *et al.* (2005) and Grenville-Briggs & Van West (2005) reviewed the upregulation of genes encoding enzymes involved in amino acid biosynthesis in both the plant and the pathogen suggesting that *Phytophthora* species require elevated amino acid production at the onset of infection, which may also refer to downy mildews such as *P. viticola*. In that case, amino acid biosynthesis could provide a potential metabolic target for chemical control.

A specific inhibitor of GS is glufosinate ammonium used as a postemergence contact herbicide. Inhibition of GS activity by glufosinate leads to a rapid accumulation of high levels of intracellular ammonia in plants. As a result, photosynthesis stops and chloroplast structure is disrupted causing plant cell death. Interestingly, as a benificial side effect, glufosinate can also reduce fungal diseases in glufosinate-resistant plants (Wang *et al.* 2003). In spite of the fact that glufosinate ammonium targets a primary pathway shared commonly by plants and saprophytic microorganisms, nearly nothing is known about its effect on microbial activities of biotrophic or hemibiotrophic plant pathogens. However, preliminary experiments revealed an antifungal capacity of glufosinate ammonium against oomycetes such as *P. viticola*, *Phytophthora infestans*, and *Pythium ultimum* (Kortekamp, 2008).

Material and Methods

Plants of the susceptible *Vitis vinifera* cv. Riesling were grown in pots filled with sand and loamy soil in the greenhouse at 20 °C and fertilised weekly. The *P. viticola* inoculum was composed of an uncharacterised mixture of isolates from previously infected grapevines from an infested vineyard in the Palatinate.

In order to investigate the effect of glufosinate ammonium on symptom development and growth of *P. viticola*, leaf discs were excised using a cork borer, placed upside down on tap water (control) or glufosinate ammonium solution (Basta®, Bayer CropScience, Frankfurt, Germany), and inoculated with a sporangia suspension. The intensity of sporulation was defined 7 days after the inoculation.

The effects of postinfectional treatments were also tested with leaf discs that were inoculated, incubated and after one to three days transferred to new Petri dishes containing glufosinate ammonium at distinct concentrations. Additionally, the development of the pathogen in inoculated leaf discs was assessed microscopically. Preparation of leaf discs for light microscopy was accomplished following Kortekamp (2005). Preinfectional treatments were performed by exposing leaf discs to glufosinate ammonium for 24 hours. Then, samples were transferred to Petri dishes with pure water and inoculated directly or one to three days post treatment.

To test if glufosinate ammonium has a direct effect on the pathogen, which could not be tested in infected leaves, where both the pathogen and the host may respond to the herbicide, sporangia of *P. viticola* were mixed with pure glufosinate ammonium (Dr. Ehrenstorfer, Augsburg, Germany) at different concentrations, and the inhibitoric effect on zoospore release and behaviour was investigated.

In addition, radial growth inhibition studies were performed using cultures of *Botrytis cinerea*, *Guignardia bidwellii*, *Penicillium expansum*, and *Phomopsis viticola*. Glufosinate ammonium was incorporated as the formulated herbicide Basta® into the appropriate agar medium.

The effect of glufosinate ammonium on grapevine plants was tested by an analysis of the chlorophyll content in leaves. For this purpose, leaf discs were used to extract chlorophylls and carotinoids at the end of the treatment period(s) (Kortekamp, 2008). Chlorophyll and pigment concentrations were determined spectrophotometrically according to the absorbance coefficients determined by Lichtenthaler (1987). To prove the existence of hormesis, a dose-response curve that include several doses below the adverse effect concentration was generated. The statistical significance of the stimulation was assessed by a regression analysis (Cedergreen *et al.* 2005).

To test if small amounts of glufosinate ammonium may interfere with must fermentation, dry yeast (Oenoferm® Rouge, Erbslöh, Geisenheim, Germany) was suspended in sterile grape juice and incubated. Aliquots were transferred to Erlenmeyer flasks, amended with glufosinate ammonium to give various concentrations, and incubated. Must density was determined daily under axenic conditions over a 10-day period.

A field experiment was conducted in order to analyse the effects of the herbicide Basta® with glufosinate-ammonium as the active ingredient on the grapevine plant and the infestation with *P viticola*. The herbicide was applied at sublethal concentrations and the toxic effects on leaves and berries based on different quality parameters were estimated.

Results

An application of glufosinate ammonium caused severe effects on growth and development of the pathogen and also on leaf vitality in a dose dependent manner. In the dose response experiments, low doses did not cause any visible negative effect on leaf samples, but high doses were unacceptably phytotoxic. No significant differences in total leaf chlorophyll concentration were found in samples treated with glufosinate ammonium at a concentration of 0.05 to 0.15 mM. Higher doses (0.3125 and 0.625 mM) led to significantly increased chlorophyll concentrations of about 2.4 ± 0.3 mM and represented a significant hormetic-stimulatory response. Hormetic effects can be found in organisms of different taxa such as animals, plants, and microbes, when exposed to different stresses, and are characterised by stimulatory responses instead of reducing the vitality.

Since an application of glufosinate within a range of 0.05 to 0.625 mM did not affect plant viability over the time period tested (≤ 7 dpi), these concentrations were selected for subsequent trials. Germination of sporangiospores and zoospore release was not affected on leaf discs floating on glufosinate ammonium regardless which concentration was used. However, spreading of the intercellular mycelium was reduced (Figure 1) also leading to a reduced sporulation in a dose dependent manner.

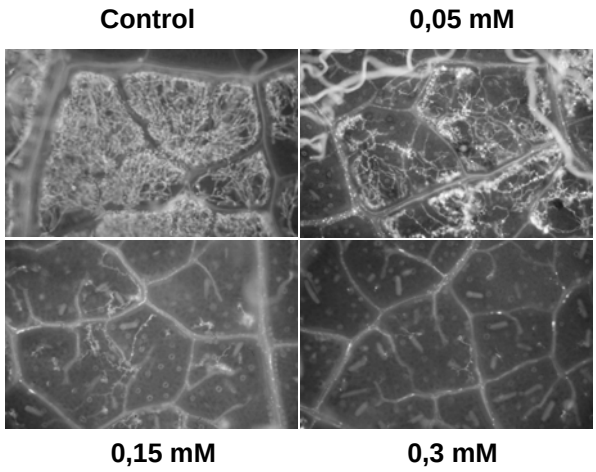

Figure 1: Mycelial growth of *P. viticola* 7 day post inoculation. Incubation of leaf discs on glufosinate ammonium led to a retarded hyphal growth in a dose dependent manner.

Pre- and postinfectional treatments also resulted in significantly reduced sporulation, but the inhibitoric effect of glufosinate ammonium on spore production decreases with increasing time intervals between treatment and inoculation. Zoospore release and movement was not affected at glufosinate ammonium concentrations up to 0.625 mM, whereas higher doses completely inhibited liberation and movement of zoospores.

Since glufosinate ammonium seems to have antifungal properties as described above, several fungal organisms putatively pathogenic to grapevine (*Botrytis cinerea*, *Guignardia bidwellii*, *Penicillium expansum*, *Phomopsis viticola*) were also exposed to glufosinate ammonium in an *in vitro* assay. The herbicidal compound caused reduction of mycelial growth in a dose dependent manner. *G. bidwellii* was the most sensitive pathogen, and mycelial growth was reduced about of 80 % when exposed to a 500fold diluted solution of glufosinate ammonium normally applied to the field. As an interesting side effect, glufosinate ammonium nearly inhibited spore production in *Penicillium expansum* cultures when exposed to the same low concentration (Albrecht & Kortekamp, 2009). Spray applications of glufosinate ammonium to the ground to control excessive weed growth could help to reduce the built-up of secondary inoculum of fungal pathogens in the same season or primary inoculum for the following year.

Since fermentation of must by yeasts may be affected due to residues of spray applications attached to grapes, the fermentation of grape juice was investigated. Fermentation was not significantly affected using glufosinate concentrations up to 0.01mM, but was significantly delayed at concentrations of more than 0.01mM. The delay of fermentation increased with increasing concentrations of glufosinate ammonium. The onset of fermentation was delayed of about one day at a concentration of 0.05mM glufosinate ammonium and of about four days at a concentration of 0.1mM glufosinate ammonium. There was no fermentation detectable at concentrations exceeding 0.1mM glufosinate ammonium. However, herbicide application, performed one or two times per year, will not lead to a reduced yield or wine quality.

In addition to the investigation of the effect of glufosinate ammonium on grapevine leaves, the downy mildew

pathogen, and other fungal pathogens *in vitro*, a field experiment using the herbicide Basta® was conducted. The herbicide was applied at sublethal concentrations and the toxic effects on leaves and berries based on different quality parameters were estimated. Low concentrations of glufosinate-ammonium (< 0,625 mM) did not affect the chlorophyll and amino acid concentrations in leaves. This was also the case for the content of salicylic acid and phenolic compounds. Furthermore, must parameters such as sugars, acids, and alcohol also remained unchanged compared to the fungicide-treated or untreated control, whereas the concentration of nitrogenous components metabolically available to yeasts increased with increasing concentrations of glufosinate-ammonium.

The amount of berries harvested at the end of the experiment depended on the glufosinate-ammonium concentration applied. The harvest was highest when glufosinate-ammonium was applied as a 0.1 mM solution and differed not significantly from the fungicide-treated control, but decreased with increasing herbicide doses. However, the infestation of leaves with *P. viticola* was significantly reduced by the application of the herbicide, even though not each infection was eradicated due to the non-systemic mode of action of the herbicide. The particular influence of the herbicide onto this host-parasite interaction still remains unclear. Since applications of glufosinate-ammonium at low concentrations seem not to affect grapevine plants negatively, but were able to reduce the infestation with downy mildew, the specific mode of action may reveal new alternatives for the control of fungal pathogens.

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Towards the identification of avirulence genes from *Plasmopara viticola*, the causal agent of grapevine downy mildew

P. Mestre, M.C. Piron, D. Merdinoglu

UMR 1131 INRA / Université de Strasbourg Santé de la Vigne et Qualité du Vin, 28 rue de Herrlisheim, BP 507, 68021 Colmar Cedex, France

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Grapevine Downy Mildew, caused by the biotrophic Oomycete *Plasmopara viticola,* is one of the most important diseases affecting vineyards. The pathogen attacks grapevines worldwide causing important economical losses. The current strategy to control the disease relies totally on the use of chemical fungicides. This practice not only is expensive (in France alone its cost is estimated to be around 150 million euro per year) but also causes a slow and progressive damage to the environment. On top of this, the arising of pathogen strains resistant to fungicides diminishes the efficiency of this practice.

An alternative to the systematic use of fungicides is the use of varieties showing resistance to the pathogen, which is cost-effective and environment friendly. However, since all cultivated European grapevine varieties are susceptible to *Plasmopara viticola*, the resistance needs to be introduced from other *Vitaceae* through breeding programmes that ensure that the agronomically and technologically important characteristics of the varieties are maintained. Breeding programmes have resulted in the creation of downy mildew resistant varieties that are currently grown on limited acreages, such as Regent and Solaris. At INRA Colmar we are developing a breeding programme for resistance to grapevine downy mildew that exploits mainly the resistance to *P. viticola* found in *Muscadinia rotundifolia*, whereas other sources of resistance are being characterised.

Efficient breeding for disease resistance requires a solid understanding of the pathosystem. In order to ensure that the resistance will be efficient and durable we need, on one hand, to understand the genetic and molecular basis of the different sources of resistance, and, on the other hand, to evaluate the genetic diversity and evolutionary potential of the pathogen populations. The last few years have witnessed progress in our knowledge about the genetic diversity of *P. viticola* populations as well as in the characterisation of the genetic basis of the resistance derived from *M. rotundifolia, Vitis riparia* and the varieties Regent and Bianca.

Despite the advances towards the characterisation of genetic basis of the resistance to grapevine downy mildew nothing is known about the nature of the corresponding avirulence genes from the pathogen. Plant disease resistance proteins recognise pathogen-encoded factors, so called avirulence genes, and trigger defense responses leading to pathogen arrest. The identification of avirulence genes is a necessary step not only to understand the biology of the interaction but also to design appropriate strategies to fight the pathogen. The analysis of variability for avirulence genes in pathogen populations is an important tool for the prediction of the evolutionary potential of the pathogen, which in turn is useful to design adequate strategies for the efficient deployment of durable resistances. Moreover, the isolation and characterisation of the avirulence genes corresponding to known resistance genes allows to estimate the durability of the resistance based on the penalty imposed upon the pathogen by the resistance gene.

Avirulence genes identified so far from Oomycetes are small secreted proteins, carrying a signal peptide and a particular RXLR motif, whose expression is induced upon infection and whose ectopic expression cause an hypersensitive response in the presence of the corresponding resistance gene. Thus, based on knowledge from other Oomycetes, *P. viticola* effectors can be identified using a candidate gene strategy based on data mining of genomic resources. Public genomic resources of *P. viticola* are very limited, not to say inexistent. A recent search at NCBI/EMBL databases produced 79 *P. viticola* entries (10 ESTs and 69 core nucleotides), the majority sequences of mitochondrial or ribosomal origin. In consequence, the identification of avirulence genes by means of a candidate gene strategy requires developing genomic resources for *P. viticola*.

Since the expression of avirulence genes is induced upon infection, we first attempted to obtain a cDNA library enriched for sequences induced upon infection using suppression subtractive hybridization (SSH): cDNAs from in-vitro germinated cysts and from chemically induced plant leaves were subtracted from cDNA from infected leaves. Using this procedure we expected to minimize the amount of pathogen-induced grapevine genes included in the library as well as the pathogen housekeeping genes. The results obtained with this strategy were disappointing: there were a very low proportion of pathogen sequences and the sequences were truncated, which makes them not suitable for a candidate-gene approach.

As an obligate biotrophe, *P. viticola* can only grow on living tissues and the pathogen biomass in the invasive stages of infection is quite low compared to the plant biomass. Nevertheless, zoospores are easily obtained by washing off sporangia from leaves at the late stages of infection. Interestingly, the first stages of pathogen development (growth of germinative tubes and vesicle formation) can be reproduced in vitro just by adding sodium chloride to an aqueous suspension of spores (Figure 1).

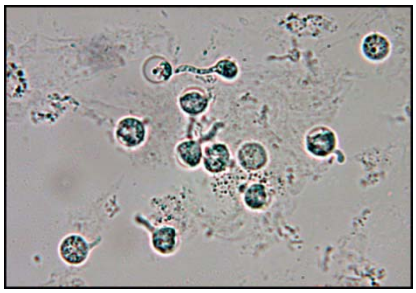

Figure 1*: In vitro* germinated spores of *Plasmopara viticola*

We obtained two cDNA libraries, one from grapevine leaves in the invasive phase of *P. viticola* infection (non-sporulating) and a second one from *in vitro* germinated zoospores. The availability of the grapevine genome sequence and the genome sequences of several Oomycetes allows us to distinguish between sequences from plant and pathogen origin. Sequencing an aliquot of clones from each library showed that 15-20% of sequences of the cDNA from infected tissue are expected to be from pathogen origin, while around 5% of sequences from the cDNA from germinated spores are from plant origin, due to contamination in the purification procedure. Sequencing 1920 clones from the library obtained from germinated zoospores resulted in the identification of 1063 nuclear ESTs (Expressed Sequence Tags) from *P. viticola*, the rest belonging to sequences from ribosomal, mitochondrial and plant origin. Sequence analysis revealed the presence of 58 ESTs from genes putatively involved on pathogenicity (secreted proteins, glucanase-inhibiting proteins, RXLR proteins, protease inhibitors, etc) and thus candidates to behave as avirulence genes.

In summary, in vitro germinated zoospores proved to be a suitable material for the identification of avirulence genes. The availability of next-generation sequencing technologies allows obtaining huge amounts of sequence data at a reasonable cost. Sequencing cDNA from germinated spores using such technologies should provide us with the genomic resources necessary to identify avirulence genes using the above-described candidate-gene based approach.

Cytological and molecular analyses of the first infection steps of *Erysiphe necator* reveal its interaction with the host plant

C. Tisch[a], L. Kern[a], N. Schmalschläger[a], G. Leubner[b], E. Bieler[c], HH. Kassemeyer[a]

[a]Staatliches Weinbauinstitut Freiburg, Dept. Biologie, Merzhauser Str. 119, 79100 Freiburg im Breisgau, Germany, [b]Albert-Ludwigs-Universität Freiburg, Institut Biologie II, Schänzlestr. 1, 79104 Freiburg im Breisgau, Germany, [c]Universität Basel, Zentrum für Mikroskopie am Pharmazentrum/Biozentrum, Klingelbergstr. 50-70, 4056 Basel, Switzerland.

Germination of the conidium and formation of the primary appressorium is a dynamic process crucial for the further development of *Erysiphe necator*. Successful propagation of *E. necator* and colonisation of plant tissues can only occur if the pathogen gains access to host cells. On the other hand the host plant can recognize the invader during penetration and haustorium formation. Early recognition of the pathogen by corresponding receptors can give rise to a defence response resulting in a cessation of the pathogen's development. Hence the processes at a structural and molecular level in both the pathogen and the host plant are of outstanding interest for an understanding of the pathogenesis and epidemic development of *E. necator*.

The course of conidium germination and appressorium formation was analysed using Epifluorescence-Microscopy (EFM), Atomic-Force-Microscopy (AFM) and Low-Temperature-Scanning-Electron-Microscopy (LTSEM). For this purpose whole plants and leaf discs of *Vitis vinifera* cv. Müller-Thurgau and V. *riparia* cv. Gloire were inoculated with a defined amount of conidia and kept under constant temperature (24°C). At distinct time intervals after inoculation (hpi), samples were taken and prepared for EFM, AFM and LTSEM. In the same manner, inoculated leaves and leaf discs were used to determine the course of the defence response directed against the invader. The transcription of putative defence genes such as PR-proteins (PRPs) as a response to a challenge infection was analysed using quantitative RT-PCR. The expression of PRPs was also quantified at the protein level.

Within 2 hpi a germination tip was observed at the distal end of the conidium. Cytological analyses with organell specific fluorescent stains for the nucleus (Hoechst 33342), mitochondria (Mito-Tracker Green FM) and endoplasmatic reticulum (ER-Tracker Red) revealed a transport of these subcellular structures to the tip of the developing primary hypha. The formation of a lobed appressorium started at the tip of the primary hypha when it reached a length of approx. 5µm at 6 hpi. The nucleus in the conidium divided and one of the nucleii migrated into the developing appressorium. At this developmental step, a high subcellular activity was indicated by a high abundance of mitochondria and a dense ER-network. At 8 hpi appressorium formation was completed and a secondary hypha emerged from the opposite side of the conidium. Up to this stage no differences in the course of the development of *E. necator* were noted between the two host genotypes. Differences between the resistant and susceptible *Vitis* genotype did not occur until there was further growth of secondary hyphae and colonisation of the host surface had commenced.

The molecular analysis of the defence response in the two genotypes showed an increasing transcription of PRPs such as a *Vitis* β-1,3-glucanase and a *Vitis* class III chitinase in response to the challenge infection by *E. necator*. In the same way, a lipoxygenase involved in defence signalling was activated by the pathogen. In both genotypes the defence response was activated during the first stages of infection and colonisation by *E. necator*. The results indicate that a delayed and inefficient expression of the defence mechanisms may be responsible for susceptibility of *Vitis* genotypes to *E. necator*.

Characterization of the necrosis producing protein NPP_{PV} from *Plasmopara viticola* belonging to the Nep1-like protein family (NLPs) and its putative role in the host-pathogen-interaction

J. Fahrentrapp[ab], T. Seibicke[a], H-H. Kassemeyer[a]

[a]Staatliches Weinbauinstitut Freiburg, Dept. Biologie, Merzhauser Str. 119, 79100 Freiburg im Breisgau, Germany; [b]present address: Plant Pathology, Institut f. Integrative Biologie, Universitätstrasse 2, 8092 Zürich. Switzerland.

Recently the role of oomycete-derived necrosis and ethylene inducing peptide 1-like proteins (Nep1-like proteins or NLPs) as a triggers of a multiple defense response in *Arabidopsis thaliana* has been described (Fellbrich *et al.* 2002, Qutob et al 2006). Proteins from the NLP family have been found in oomycetes, fungi and bacteria, but not in plants and animals. In plant tissue, NLPs initiate programmed cell death and as a consequence the formation of necrosis. To date the occurrence of NLPs is exclusively described in organisms with a necrotrophic or hemibiotrophic lifestyle.

We cloned and sequenced a putative Nep1-like protein from *Plasmopara viticola.* Sequence analysis revealed a homology to the sequences of NLPs from oomycetes, fungi and bacteria published in the data base. This is the first time that a NLP is described from an oomycete considered to be an obligate biotrophic pathogen. To elucidate the role of this necrosis producing protein (NPP_{PV}) from *P. viticola* we have studied its expression in *Vitis* genotypes during the infection and colonization of the host tissue. For this purpose samples were taken at distinct time intervals after inoculation from leaf discs inoculated with a defined amount of *P. viticola* sporangia. Following the extraction of total RNA and cDNA synthesis we quantified the abundance of the NPP_{PV} transcript by means of Real-Time PCR. Simultaneously the course of the pathogen development in the host tissue was analyzed microscopically using fluorescence stains to detect *P. viticola* within the host tissue. To relate the transcriptional activity to the mycelium mass in the host tissue we estimated the quantity of the pathogen by means of Real-Time PCR with specific primers for *P. viticola.* The quantitative PCR revealed a rapid increase of the NPP_{PV} transcription within the first 12 hours post inoculation (hpi). In this time interval *P. viticola* penetrates the host *via* the stomata and establishes in the substomatal cavity underneath the guard cells. At 6 hpi when the substomatal vesicle was formed a maximum of NPP_{PV} transcript expression was observed, while no transcription occurred from 42 up to 90 hpi. We detected a second increase of NPP_{PV} transcription at 96 hpi when secondary vesicles and the initials of sporangiophores were formed in the substomatal cavity. To get more insight in the expression of this Nep1-like protein from *P. viticola* during the infection and colonization cycle we produced antibodies against $NPP_{PV.}$ For the antibody production we used a recombinant NPP_{PV} antigen. The western blot analysis revealed a high affinity of the antibody to the recombinant protein.

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Sporulation in *Erysiphe necator*: Signals, differential gene expression and possible implications for disease management

L. Wakefield[a], L. Cadle-Davidson[ab], DM. Gadoury[a], R.C. Seem[a]

[a] Department of Plant Pathology and Plant Microbe Biology, Cornell University, Geneva, NY 14456, USA;
[b] USDA-ARS, Grape Genetics Research Unit, 630 W. North St., Geneva, NY 14456, USA;

Abundant production of conidia is a driving factor for epidemics of grape powdery mildew (*Erysiphe necator* (syn. *Uncinula necator*). Developing strategies for reducing asexual sporulation may therefore have considerable impact on disease spread. Previous investigations revealed that temperature and humidity influence the production of conidia (Delp, 1956; Carroll and Wilcox, 2003), established evidence for a signal that coordinates the onset of asexual reproduction, and suggested that signal production is influenced by light and colony density (Gadoury *et al.*, 2010). The genetic basis for these signals in powdery mildews had not been previously described. Significant work in *Aspergillus nidulans* had established signaling pathways for developmental control (Fischer and Kües, 2006). These studies and related investigations in *Neurospora* and *Penicillium* have revealed roles for light signaling, heterotrimeric G proteins and coordinated production of asexual spores and secondary metabolites (Kasuga and Glass, 2008; Calvo, 2008; Garcia-Rico *et al.*, 2008, Yu *et al.*, 2006). The implications of these basic models for control of asexual sporulation in *E. necator* are unknown however. Previous studies in *E. necator* have established a basic model for the timing of asexual sporulation events. Following inoculation, *Erysiphe* colonies grow in a purely vegetative state for a period of 5-9 days. Conidiophores then appear singly and in groups throughout the body of the colony, suggesting the presence of some signal which coordinates asexual development. This pattern of purely vegetative growth followed by asexual sporulation indicates that *Erysiphe* may have the same need to acquire developmental competence before sporulation in a manner analogous to that observed in *Aspergillus* (Mooney and Yager, 1990). It may also be that *Erysiphe* contains some repressor of asexual and sexual development such as FadA that keeps the colonies in a purely vegetative state. Unlike *A. nidulans*, *Erysiphe* is bipolar heterothallic and sexual reproduction requires pairing of compatible mating types (Gadoury and Pearson, 1991). Observational data suggest that asexual sporulation is repressed upon commencement of mating. When colonies of opposite mating type are inoculated in close proximity on a leaf, a zone of conidiation inhibition appears between the colonies as the hyphae merge, and eventually asexual sporulation is entirely switched off across both colonies. In established colonies in the field, the arrival of a second mating type results in suppression of production of fresh conidia and eventually extant conidiophores shrivel.

The goal of this research was to identify sequences differentially expressed during vegetative growth and throughout asexual and sexual reproduction in *E. necator*. We used cDNA-AFLP analysis to identify sequences associated with the developmental stages. Our investigations show some overlap between sporulation-associated sequences in *E. necator* and other model systems, but also indicate the fungus may substantially rely on unique genes.

Material and Methods

Plant material, inoculum and pathogen growth. Seedlings were grown from seeds collected from open pollinated Chardonnay and Riesling vines according to Ficke *et al.* (2003) and were inoculated at the 5-6 leaf. Two isolates of *E. necator*, 10-18 and 10-36, of opposite mating type were collected from New York vineyards and grown separately on seedlings under the same conditions. For growth of the asexual stages, conidia were harvested from colonies approximately 8 days post inoculation (dpi). Suspensions of each isolate were made separately and normalized to a concentration of 200 conidia per 5 µl drop. For colonies where sexual reproduction was desired, conidial suspensions of both isolates were prepared as above, mixed in equal proportion. Colonies for RNA extraction were prepared by placing multiple 5 µl drops of the conidial suspension across the entire surface of the youngest fully expanded leaf of a grape seedling. When the colonies reached the desired stage, all inoculated leaves were observed under a dissecting scope for uniformity of development. Leaves showing at least 90% uniformity in terms of relative radial growth and appearance of the appropriate sporulation stage were selected for RNA extraction.

RNA Isolation. RNA was extracted from each of four stages: pre-sporulation (3 dpi), at conidiophore initiation (~ 5 dpi), at full sporulation (8 dpi), and at the development of mature ascocarps (approximately 4 weeks post inoculation). All RNA extractions were performed according to Cadle-Davidson *et al.* (2009). For the three asexual stages, two independent isolations were performed from each of two isolates. For the sexual stage, four independent isolations were performed. Within each stage, equal amounts of total RNA were pooled from the four independent extractions to form one pool/stage of interest. mRNA was isolated from total RNA using the PolyATtract system (Promega, Madison, WI). The mRNA was not eluted from the beads in the final step, and the beads were used directly in the iScript™ cDNA Synthesis Kit (Biorad, Hercules, CA) to convert the mRNA into cDNA.

cDNA-AFLP Analysis. The cDNA-AFLP protocol was adapted from Bachem *et al.* (1996). cDNA from each stage was digested with TaqI followed by an AseI digestion. Resultant fragments were then ligated to adapters for amplification. Pre-amplification was performed with a TaqI+0 primer. Five µl of the diluted pre-amplifiction product were used as template for the selective amplification which was carried out with 45 primer combinations using two selective nucleotides on each primer. Selective amplication products were

separated by running on a 5% polyacrylamide gel in a BioRad Protean II xi Gel System (Hercules, CA). Following separation, fragments were visualized by silver staining according to the protocol by Echt *et al.* (1996). Fragments of interest were picked from the gel and eluted, and 1 µl of which was used as a template for reamplification using the pre-amplification primers. Samples with visible, single bands were purified and Sanger sequenced directly using the pre-amplification primers at Cornell University's Core Laboratories Center.

Sequence Analysis. Homology searching by blastx and tblastx was carried out against the NCBI database. Longer length sequences for the amplified products were found by performing a nucleotide BLAST of each sequence against a conidial stage cDNA library database containing over 32,000 contigs from Roche-454 sequencing of *E. necator* isolate Geneva-14 (G14) (Cadle-Davidson, unpublished data). The cDNA contigs matched in this search were then searched against the NCBI database using blastx and tblastx.

Real Time PCR Confirmation. To confirm differential expression of the identified sequences, quantitative RT-PCR (qRTPCR) was performed. RNA was isolated from colonies at the 3 dpi, conidiophore initiation, full sporulation, and ascocarp initiation stages. Extraction of the RNA, purification of mRNA and synthesis of cDNA was performed as described above. Each pool was then normalized to equal cDNA concentration. Following purification, the cDNA samples and remaining mRNA were analyzed with *E. necator* primers to confirm the absence of *E. necator* genomic DNA.

After analyzing the sequence data from the cDNA-AFLP experiment, 17 sequences of interest were identified based on their expression pattern and match in the BLAST searches. Primers for real time were designed for all 17 sequences and tested for quantitative response to gDNA and specificity by melt curve analysis. Three technical replications were performed on all melting curves. Following this initial analysis, six sequences were selected for further analysis based on their melting curves. Three replications of cDNA from each time point were run for each gene of interest and original concentration were estimated based on the standard curve.

Results

A total of 45 primer combinations were used for cDNA-AFLP analysis, giving 21-64 bands, or transcript derived fragments (TDFs), ~100-800 bp in size, per primer combination. Across all 45 primer combinations a total of approximately 1,600 TDFs were visualized. Within these, 620 TDFs (39%) were polymorphic across one or more of the four stages, suggesting differential expression. Of these polymorphic bands, 242 were directly sequenced. The remaining bands out of the 620 TDFs either gave poor product upon reamplification or gave a degraded product when sequenced. When searched against the NCBI database using blastx and tblastx, 45 (20%) of these had a significant match (E value $\leq$ 1 E-03). Only one sequence matched to *Vitis vinifera*, indicating the extraction method was successful in targeting *Erysiphe* specific RNA. To improve the ability to find matches, the short length TDFs were searched against an *E. necator* cDNA library. Of the 242 sequences searched against the library 162 (67%) showed significant matches to 1-9 cDNA contigs. There were 121 contigs that showed a significant match when searched against the NCBI database, again using blastx and tblastx. Where the search of the original TDF had matched multiple contigs in the cDNA library, the group of matching contigs matched similar sequences in the NCBI database, indicating that these multiple hits were members of a gene family.

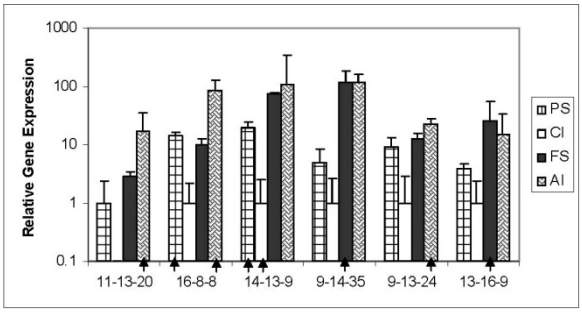
Functional classification of sequences. Based upon searches of the relevant literature and analysis of the sequences, five classes of differentially expressed fragments were identified. Of the *E. necator* sequences assigned to each class, 45% of the sequences had no significant match in the databases searched. An additional 16% matched hypothetical fungal protein sequences of unknown function. Among the sequences that matched sequences of predicted or known function, the largest class, representing 13% of differential transcripts, were sequences involved in metabolism. Other groups of interest, each representing 2-8% of the TDFs, included transcription factors, signaling proteins, and transport proteins.

Confirmation of differential expression by real-time RTPCR. Normalized expression confirmed that the cDNA pools were free of contaminating genomic DNA. The results from five of the six sequences showed good agreement with what was expected from the cDNA-AFLP analysis (Figure 1). Where expression was expected to be highest at full sporulation, expression at ascocarp initiation was also high. In general, expression was lowest at presporulation and conidiophore initiation. The single gene with expected expression in the early stages, 3182, showed low expression at pre-sporulation and conidiophore initiation and high expression in later stages.

Discussion

The cDNA-AFLP method of analyzing transcriptional regulation across developmental stages has been described as useful for comparisons across multiple treatments in non-model species (Polesani *et al.*, 2008) and particularly appropriate in studies of obligate biotrophs where little sequence info is available (Van der Biezen *et al.*, 2000). Our results demonstrated similar utility in *E. necator*. Using 45 primer combinations, we visualized 620 differentially expressed fragments, out of 1,600 total fragments. This level of differential expression may reflect the large physical changes attending the progression from vegetative to either asexual or sexual reproductive growth, in addition to simultaneous expression related to host infection. Of the sequenced fragments, 45% showed no significant homology to known sequences, underscoring the appropriateness of using a platform where prior sequence knowledge was not required. Analysis of those sequences which showed similarity to previously described sequences revealed several patterns in gene expression: metabolism; signaling, transcription regulation; cell membrane/transport; and protein maintenance/degradation.

Our results have shown some similarity between control of development in *E. necator* and the models from *Aspergillus* and *Neurospora*, particularly in the areas of G-protein signaling, transcriptional regulation and nuclear transport.



TDF Code	Expected Expression	Annotation
111320	Ascocarp Initiation	Acetyl CoA FacA Synthase
1688	Presporulation, Ascocarp Initiation	MSF1 Domain Protein
14139	Presporulation, Conidiophore Initiation	C2H2 Zinc Transcription Factor
91435	Full Sporulation	Pianissimo
91324	Ascocarp Initiation	Catalase peroxidase
13169	Full Sporulation	FBox Domain Protein

Figure 1: qRT-PCR analysis for 6 selected genes across four time points in *E. necator* sporulation. Expected expression was based on the cDNA-AFLP analysis. The stages are as follows from left to right: pre-sporulation (PS), conidiophore initiaton (CI), full sporulation (FS) and ascocarp initiation (AI). All data were normalized to one expression unit. Data represent fold change of gene expression across all four time points. The stage with the highest expression is marked with an ↑. Bars represent the standard error calculated on 3 technical replicates.

The largest group of sequences, however, was those with no match to known sequences. Forty-five percent of sequenced TDFs showed no significant match. This indicates that while control of sporulation in *E. necator* may share some basic elements with established systems, it is likely that there are significant points of divergence as well. Given the differences in growth and reproduction between the powdery mildews and the model fungi, this is not surprising. Most significantly, the powdery mildews are obligate biotrophs. It seems likely that they have a more intimate connection with their host species and may rely on the host for developmental cues in ways fungi like *Aspergillus* do not. In addition, *E. necator* is heterothallic unlike the self-fertile *A. nidulans*. Sexual reproduction is initiated upon contact between hyphae of opposite mating type, not in response to environmental triggers such as lack of light. It is possible that control of asexual versus sexual development is handled by differing complexes.

Functional analysis of genes as well as potential isolation of chemical triggers or suppressors of sporulation will potentially open up new means of disease control for grapevine powdery mildew. If the suppressors are amenable to large scale manufacture, they may be directly applied in a spray. It could also be that transgenic grapes with the potential to interfere with signaling processes may be created. Given the nature of the wine industry and the resistance to genetically modified crops in critical portions of the market, it may also be that the identification or the creation of a biocontrol with the ability to interfere with signaling may be a better avenue for disease control. Potentially, a combination of the above techniques could be used, providing a multi-level strategy for repressing sporulation and therefore interfering with disease spread. Such a strategy may prove the most effective means for controlling a pathogen like powdery mildew which shows such adaptability in overcoming disease control efforts and which can cause such significant damage at low levels of infection.

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Session 1: Posters

Quantification of stilbenes in *Vitis* spp. genotypes with different levels of susceptibility to *Plasmopara viticola* infections

S. Boso[a], K. Gindro[b], M.C Martínez[a], H-H Kassemeyer[c]

[a]Misión Biológica de Galicia. Consejo Superior de Investigaciones Científicas (CSIC), Apartado de Correos 28, 36080 Pontevedra (Spain). susanab@mbg.cesga.es; [b]Abt. Station de recherche Agroscope Changins-Wädenswil ACW Case postale 1012, CH-1260 Nyon 1;[c]Staatliches Weinbauinstitut Dept. Biology Merzhauser Strasse 119 D-79100 Freiburg im Breisgau

Plasmopara viticola is the causal agent of grapevine (*Vitis vinifera* L.) downy mildew, a major disease that can cause severe losses in yield and wine quality. Although some European grapevine cultivars show a reduced susceptibility for downy mildew, most are susceptible to this pathogen. Different efforts are being made to develop alternative protection strategies by using less susceptible cultivars. The mechanisms of resistance in *Vitis* are complex. For example, the synthesis of phytoalexins and phenolic compounds plays an important role (Langkage *et al.*, 1977; Hain *et al.*, 1993; Feys and Parker, 2000; Coutos-Thévenot *et al.*, 2001; Pezet *et al.*, 2003; 2004a,b; Gindro *et al.*, 2003). Resistant plants can transform resveratrol to products, which are more toxic for the pathogen, whereas susceptible cultivars do not accumulate viniferins but a glycosylated form of resveratrol knows as piceid, which is not toxic for the pathogen even at high concentration (Pezet *et al.*, 2004). Dercks and Creasy (1989) studied the role of ε-viniferin in the grapevine resistant degree to P. viticola. Recently, δ-viniferin, a resveratrol dehydrodimer produced by the oxidative dimerization of *trans*-resveratrol, was identified as a major stilbene in grapevine leaves infected by *P. viticola* (Pezet *et al.*, 2003). The possible role of these phytoalexins in the degree of resitance observed in *Vitis* spp. was determined by measuring the levels of viniferin in the sites of inoculation at different times after inoculation (6hpi, 24hpi, 48hpi and 72hpi).

Material and Methods

Four *V. vinifera* L. cultivars (cv. Tempranillo, cv. Cabernet Sauvignon, cv. Pinot noir and cv. Touriga National), representing more or less susceptible genotypes to *Plasmopara viticola* infections (Boso *et al.*, 2007), and one resistant *Vitis* species (*Vitis riparia* cv. Gloria de Montpellier) were studied.

A population of *Plasmopara viticola* was obtained from naturally infected plants in the experimental vineyards of the Swiss Federal Research Station of Changins (Nyon) and maintained on *V. vinifera* cv. Chasselas grown in the greenhouse. Sporangia were raised in order to prepare an inoculum following the method of RUMBOLZ *et al.* (2002). For this purpose the adaxial leaf surfaces of the plants were sprayed with a suspension of 40,000 sporangia ml^{-1} in distilled water and covered overnight with a wet polythene bag. The inoculated plants were kept under high relative humidity (RH) (>96%) overnight at 24°C. After incubation for 5 to 6 days under ambient greenhouse conditions, the plants were again maintained under moist conditions overnight to induce sporulation. From the sporulating lesions, freshly developed sporangia were collected in centrifuge tubes using a small paintbrush. The inoculum was prepared by counting thes sporangia using a Fuchs-Rosenthal chamber and adjusting to a dilution of 25,000 sporangia ml^{-1}.

At different intervals of time (6hpi, 24hpi, 48hpi and 72hpi), three pieces of leaf corresponding to the droplet surface, were cut from each inoculated leaf. Three replicates and duplicate experiments were made for each cultivar. Leaf samples were weighted and placed in a microfuge tube (1,5ml) and 60ml of MeOH were added. The tightly closed tubes were placed in a thermo-regulated shaker at 60ºC for 20 min. The methanolic extracts (30□l) were analyzed for stilbenes as described by Pezet *et al.*, (2003). Results are expressed as means in nmol.mg1 FW.

Statistical analysis: ANOVA was performed on the quantitative data recorded for the different times and genotype type. The GLM method of the SAS System v 9.1 software package was used to determine whether any differences between *Vitis* spp. genotypes at different times were significant. Fisher's protected least significant differences test was performed for each fixed factor and its error.

Results

Significant difference (P<0.001) were observed in the quantitative level of stilbenes within the different cultivars, times and cultivar*time interaction (Table 1). Susceptible cultivars accumulate preferentially piceid (tr- and cis-piceide, a glycosylated forms of resveratrol non toxic product for the pathogen even at high concentration). At 6hpi resveratrol was present in *V. riparia* as well as low concentrations of ε- and δ-viniferin (<3µmol-1FW). At 24 hpi, the most susceptible Tempranillo cultivar and the resistant *V. riparia* had similar levels of resveratrol and bigger δ-viniferin. Concentrations of viniferins were low for all susceptible cultivars, whereas ε-viniferin was already importantly in *V. riparia*.

At 48 hpi, *V. riparia* showed a concentration of ε- -viniferin approximately of 168µmol mg-1 FW and lowest levels of δ-viniferin. Susceptible cultivars were found to have levels of <12µmol mg-1 FW of viniferin at the site of inoculation, whereas they had a higher concentration of piceide. Significant differences were observed for ε- and δ-viniferin in the most susceptibles cvs.(Tempranillo and Touriga), in comparison to the least susceptibles (cvs. Cabernet Sauvignon and Pinot noir).

V. riparia at 72hpi, contain a very high concentration of ε- and δ-viniferin in comparison with the rest of the cultivars. Touriga Nacional differs from others cultivars by presenting the lowest values of ε -viniferin (< 35µmol mg-1 FW). Pinot noir had moderate concentrations of δ-viniferin and had high levels of resveratrol. Pterostilbene was only detected in cv.Cabernet Sauvignon at 72hpi.

Discussion

The presence of high levels of *trans*-resveratrol in the necrotic tissues in *Vitis riparia* (72hpi) might restrict the hyphal development of *P. viticola*, as already have been said by Dai *et al.*, (1995) in resistant varieties of grapevine. Recently, Montero *et al.*, (2003) reported that in *B.cinerea* infected grapes, ethylene emission rises after 48h when the *trans*-resveratrol starts to decrease irreversibly, whereas in non-infected grapes high *trans*-resveratrol content corresponds to a low ethylene emission.

Stilbene synthesis is induced in grapevine by inoculation with the pathogens *B. cinerea* or *P. viticola* (Blaich and Bachmann 1980, Langcake 1981). Pezet *et al.*, (2004) reported that the different stilbenes are toxic for zoospores of *P. viticola*. This could affect the disease development in grapevine. High concentrations of stilbenes at the site of infection in resistant varieties as Solaris are correlated with the inhibition of *P. viticola* development. In contrast, the observed resistance in different grapevine varieties to *P. viticola* may be associated with some factors other than stilbenes (Dercks and Creasy, 1989). In this paper the fact that at 24 hpi, the most susceptible genotype (Tempranillo) and the resistant one (*V. riparia*), had similar levels of resveratrol and higher δ-viniferin, indicates that the concentration of this stilbene at that time is not correlated with the resistance level. However, the concentrations of viniferins were low for all susceptible cultivars, whereas ε-viniferin was already important in the resistant *V. riparia*. At 48 hpi and 72hpi, the quantitative and qualitative analyses of stilbenes were more representative in the resistant cultivar, because *V. riparia* showed very high concentration of ε- and δ-viniferin in comparison with the rest of cultivars. Kortekamp *et al.* (1998) reported that fungal development was stopped three days after inoculation in resistant cultivars, whereas infection of leaves was similar to that observed in susceptible cultivars. This indicates that after the beginning of infection, phytoalexins might be accumulated in resistant cultivars, which stops further development and late infection. Boso et al (2008) suggest that the zoospores germination and penetration are not simultaneous. Therefore, zoospores can try to penetrate but they might be stopped by the phytoalexins accumulation induced by an earlier penetration event. This might prevent further infection.

One more time it is very clear what is the role of the phytoalexins in the resistant genotypes, although there are still a lot of questions to be solved related whithin the susceptible genotypes.

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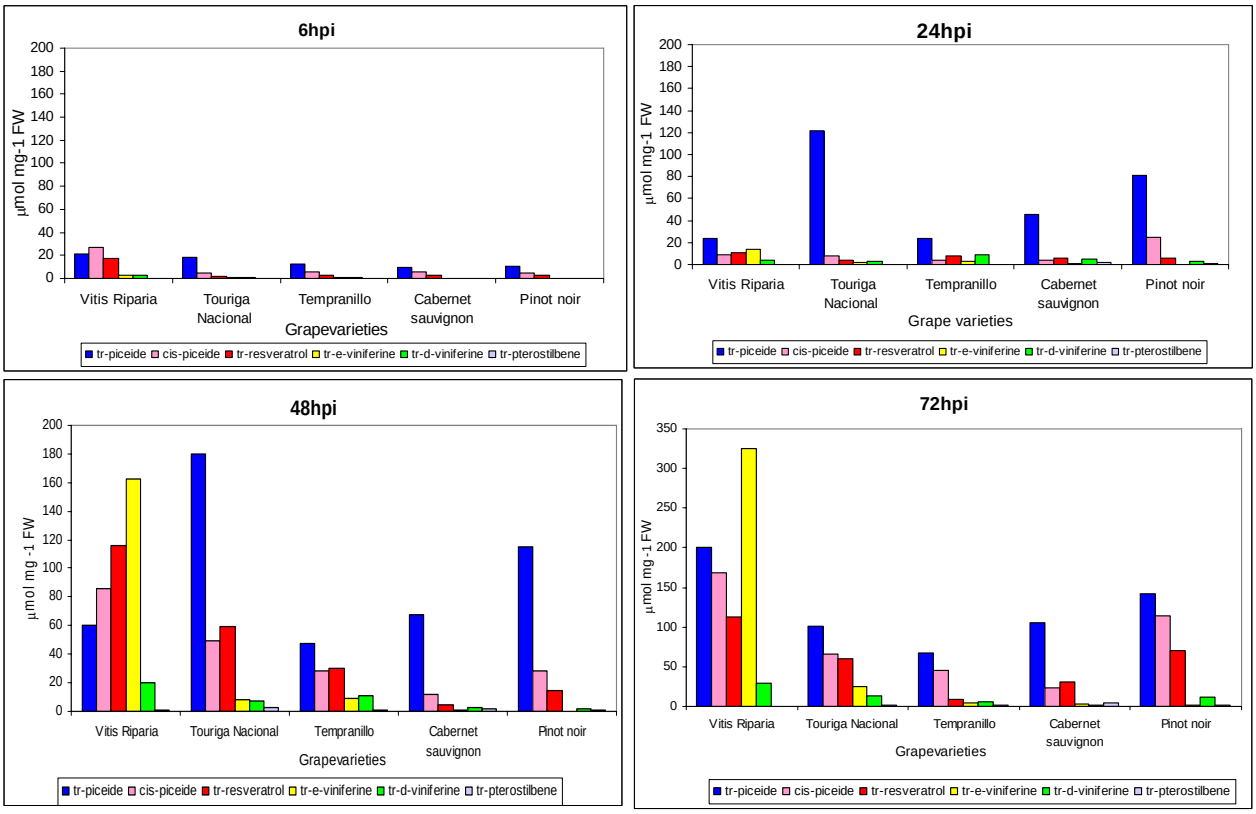

Figure 1. Qualitative and quantitative analysis of stilbenes in leaf samples as *Vitis* spp. genotypes with different susceptibility and resistance to downy mildew and at different times (6, 24, 48 and 72) after inoculation with *Plasmopara viticola*

European Wild Grapes - Genetic Relations and Susceptibility to Fungal Pathogens

S. Schröder[a], A. Kortekamp[b]

[a]KIT, Karlsruhe Institute of Technology, Botany I, Kaiserstraße 2, 76131 Karlsruhe, Germany;[b]DLR, Dienstleistungszentrum Ländlicher Raum, Breitenweg 71, 67435 Neustadt, Germany
(email: stephan.schroeder@kit.edu;andreas.kortekamp@dlr.rlp.de)

Vitisvinifera L. ssp. *silvestris* (Gmelin) Hegi, the European Wild Grapevine and ancestor of cultivated grapevine varieties (*V. vinifera* L. ssp. *vinifera*) is the sole wild grapevine species existing in Europe. This important Crop Wild Relative (CWR) species is a highly endangered species and present in only very few residual habitats. Moreover, since these habitats are often close to cultured grapes, this CWR species is prone to hybridization with its descendant crop and naturalized American and Asian rootstocks that originate from viticulture (Arrigio& Arnold, 2007)
For this reason we were interested in two questions: To what extent are the remaining *Vitissilvestris* accessions from southern Germany autochthonous and free from hybridization with grapes cultured in the surrounding area. Secondly, what is the potential of this CWR species as a genetic resource for breeding in relation to several grapevine diseases such as powdery mildew (*Erysiphe necator*), downy mildew (*Plasmopara viticola*), and black rot (*Guignardiabidwellii*).

To answer these questions we compared eight SSR markers (VVS2 (Thomas & Scott, 1993), VVMD07 (Bowers *et al.*, 1996), VVMD25, VVMD27, VVMD28, VVMD32 (Bowers *et al.*, 1999), VrZag62 and VrZag79 (Sefc*et al.*, 1999) from 29 *Vitissilvestris* plants collected from the peninsula 'Ketsch' in the Rhine river from southern Germany and seven cultured grapevine species such as 'Cabernet', 'Riesling', 'Chardonnay', 'Pinot noir' and 'Pinot blanc', Traminer and Silvaner (Figure 1).

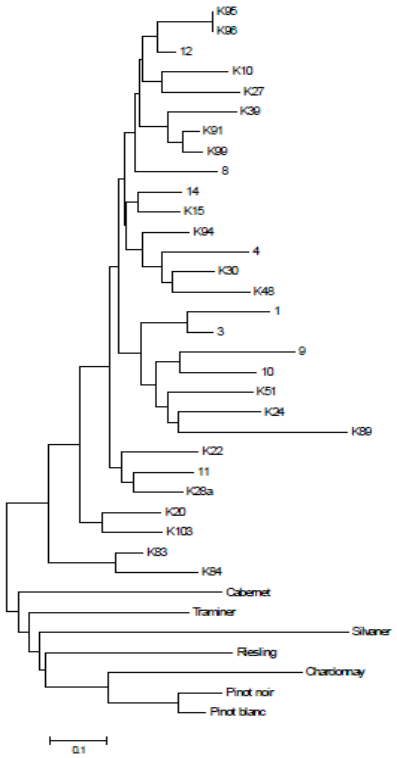

Figure 1:Phylogenetic tree of 29 *Vitissilvestris* species and seven cultured grapes created from eight SSR markers.

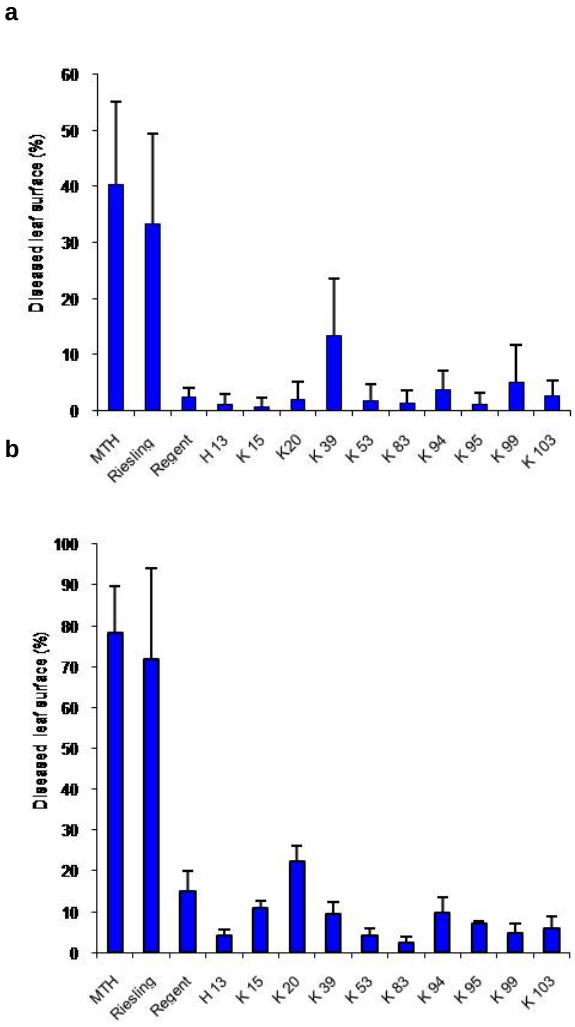

Figure 2: Percentage of infected leaf surface with: a) *Plasmoparaviticola* and b) *Erysiphenecator* of *Vitissilvestris*species in comparison to susceptible grapevine Müller-Thurgau (MTH) and Riesling, and the resistant variety Regent.

Additionally the response towards important and widespread grapevine diseases was evaluated. For this purpose, entire plants (in case for powdery mildew and black rot) or leaf samples (in case for downy mildew) were inoculated with the respective pathogen and incubated under appropriate conditions. After an incubation period of 7 days (downy mildew) or 14 days (powdery mildew and black rot), disease severity was evaluated and determined mainly as diseased leaf surface. We observed that the Wild European Grapevine accessions clustered separately from cultivated grapes, suggesting that introgression by cultured grapes from geographical closely located vineyards does not play an important role. Additionally, wild grape plants show significant resistance to a downy mildew population isolated in the Palatinate, which equals that of the resistant grapevine cultivar 'Regent' (Figure 2a). Furthermore, most wild grape plants exhibit also considerable resistance against powdery mildew (Figure 2b). These findings are surprising, since these presumably autochthonous, European wild accessions did not co-evolve with these two American pathogens such that induced resistance is not very likely. Even though the response of these wild grapes towards several strains of both pathogens isolated from different locations has to be tested in order to confirm their obvious resistance features, the possibly preformed resistance of European Wild Grapes might be due to morphological traits. Internal stomatal rims, for instance, had been found to correlate with successful arrest of pathogen colonization in several wild species of grapevine in case for downy mildew (Jürges*et al.*, 2009).

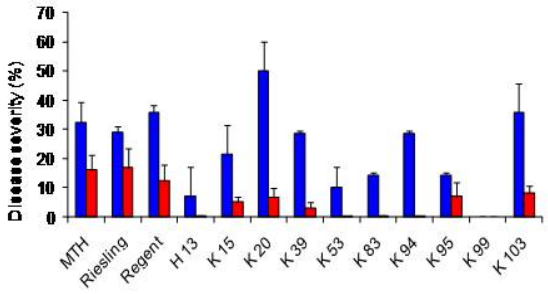

Figure 3: Severity of *Guignardiabidwellii*infection of wild *Vitis* species in comparison to cultured grapevine species MTH, Riesling and Regent (blue = diseaseseverity; red = diseaseincidence).

An inoculation of wild grapes with the black rot pathogen lead to typical symptoms in some of the plants, but did not cause any severe damage on five of ten wild grape accessions tested (Figure 3). The resistance response of these grapes was not determined in detail. However, the resistance response against all pathogens tested will be investigated in the near future using microscopical, biochemical, and molecular techniques.

In conclusion, we suggest that the protection of this endangered species is not only highly valuable for sustaining an intact ecosystem, but also for breeding purposes to enhance plant resistance of cultivated grapevine varieties in Europe.

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Proteomic characterization of grapevine induced systemic resistance activated by *Trichoderma harzianum* against grapevine downy mildew

M. C. Palmieri[a], M. Perazzolli[a], V. Metafora[b], A. Bachi[b], I. Pertot[a]

[a] IASMA Research and Innovation Centre, Fondazione Edmund Mach (FEM), 38010 San Michele all'Adige (TN) Italy, mariacristina.palmieri@iasma.it; [b] Biological Mass Spectrometry Unit DIBIT, San Raffaele Scientific Institute, 20132 Milano, Italy

Several biocontrol agents and chemicals were described for their ability to activate grapevine defense mechanisms and increase plant resistance against pathogens.

Trichoderma spp. are ubiquitous filamentous fungi and can colonize rhizosphere and phyllosphere, promoting plant growth and antagonize plant-pathogens (Vinale *et al.*, 2008). Because of this ability, several *Trichoderma* spp. are effective biocontrol agents against numerous foliar and root pathogens. The antagonistic mechanisms of *Trichoderma* spp. against pathogens include competition, production of antifungal compounds, direct parasitism of pathogens and induction of plant systemic and localized resistance (Brunner *et al.*, 2005). Physiological studies and transcriptomic analyses revealed that induced systemic resistance (ISR) is usually associated to priming state without metabolic cost for the plant (van Hulten *et al.*, 2006). Primed plants do not activate the defense-related genes, but respond to pathogen attack through an earlier and stronger defense reaction once infection occurs (Conrath *et al.*, 2006).

In the last years we demonstrated the capability of *Trichoderma harzianum* T39 to protect susceptible grapevine cultivars against downy mildew under greenhouse conditions. *T. harzianum* T39-mediated resistance is not caused by a direct effect on *Plasmopara viticola* sporangia or sporangia germination, but it is mediated by the activation of grapevine defense mechanisms (Perazzolli *et al.*, 2008). Preventive *T. harzianum* T39 treatment reduces disease severity on grapevine, similarly to what observed for the chemical inducers benzothiadiazole (BTH). BTH has no direct activity against pathogens, but increases crop resistance to diseases caused by viruses, bacteria and fungi by activating systemic acquired resistance (SAR) signalling pathways. *T. harzianum* T39 (Trichodex, Makhteshim Ltd., Israel; 8 g/l) and BTH (Bion, Syngenta Crop Protection, Italy; 1 g/l) were applied on both surfaces of basal leaves of grapevine cv. Pinot Noir, in three consecutive days prior inoculation. Plants were sprayed with water as control. *P. viticola* was then sprayed on abaxial leaf surfaces of all grapevine leaves, and the disease severity was assessed after ten days on treated (local effect) and untreated (systemic effect) leaves. Downy mildew severity was significantly reduced on *T. harzianum* T39-treated and BTH-treated (local) grapevine leaves (Figure 1). The reduction was more severe in BHT-treated plants, where we observed 93% of efficacy in comparison to 51% of efficacy in *T. harzianum* T39-treated leaves (Figure 1A).

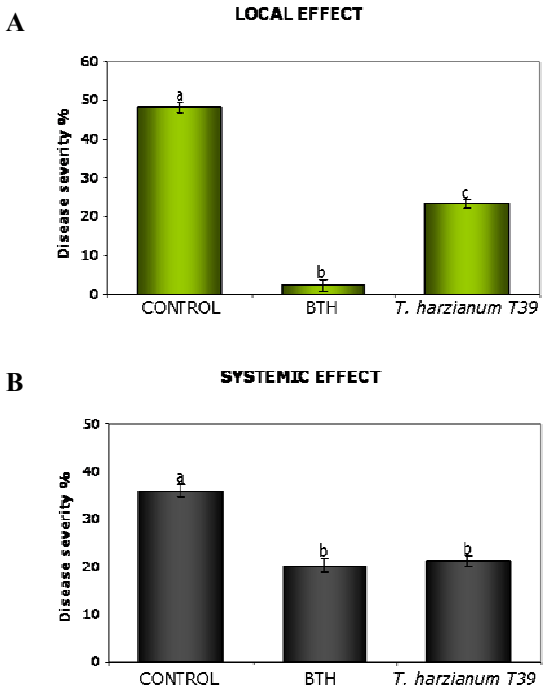

Figure 1: Disease severity of treated (local, **A**) and untreated leaves (systemic, **B**) of water (control), BTH or *T. harzianum* T39 treated plants. The mean severity and standard errors of twelve replicates for each treatment are reported. Different letters indicates significantly different data (Tukey test, $P<0.05$).

A reduction of severity was also present on untreated leaves of BTH and *T. harzianum* T39 treated plants, demonstrating the activation of a systemic resistance (Figure 1B). The systemic efficacy against downy mildew disease was similar (45% of disease reduction) in both BTH and T39-treated plants and it had lower levels compared to treated leaves on the same plant, suggesting a stronger local activation of the plant-mediated resistance mechanisms. *T. harzianum* T39 may offer a valid alternative to chemical pesticides; however more knowledge of the mechanisms is required in order to reach economically acceptable levels. Since the ISR inducers are mediating a time-dependent response that activates the alert, probably there is a continuous synthesis of cellular factors with important functions in defense responses. Therefore, we have undertaken a proteomic approach to identify the processes involved in grapevine self protection induced by T39 against *P. viticola*.

Because of the high content of polyphenols, pigments, polysaccharides and lipids in grapevine leaves, the first task was the optimization of protocols for grapevine

proteomics. In fact, secondary metabolites can severely affect the performance of protein extraction and separation. To remove these interfering substances different methodologies were compared. In the first method, the removal of phenolics from plant extracts was achieved using water-insoluble polyvinylpolypyrrolidone (PVPP), which forms hydrogen bonds with phenolic compounds. In the second method, finely ground plant tissue was subjected to 10% w/v TCA/acetone (plus 0.07% 2-mercaptomethanol) precipitation. After extensive organic solvent cleanups, the tissue pellet resulted very light coloured, indicating the removal of the majority of secondary metabolites (e. g., phenolics, pigments). A phenol extraction followed by ammonium acetate/ methanol precipitation was also used to partition proteins and lipids.
Comparison of results (from different extraction methods) demonstrated that the TCA/acetone-based precipitation was the best protocol in terms of quality and quantity of the proteins obtained, minimizing protein degradation and the presence of interfering compounds.

Proteins expressed in untreated and T39-treated plants, before and after *P. viticola* challenge were then analyzed in order to identify defense proteins and cellular pathways involved in the T39-induced resistance. For this purpose, we selected the two-dimensional gel electrophoresis (2D-gel) protein separation technique, which offers a high resolution for protein separation, in combination with MS-based protein identification. The separated proteins were visualized by Sypro Ruby staining (Invitrogen, Italy), which allowed sensitive and reproducible protein detection (Figure 2). The resulting 2-DE images, which were reproduced from a minimum of three independent biological replicates, were compared using Image Master 2D 7.0 Software (GE Healthcare, Italy) in order to create specific protein reference maps (Figure 2). The subsequent identification by MS-based technique of differentially expressed proteins, allows identifying candidate proteins responsible for the T39-induced priming state of grape plants before and 24 h after *P. viticola* inoculation.
Some of these cellular components are probably involved in the activation of the mechanism that leads to the induction of ISR inducer-responsive genes; other factors will function in cooperation with elicitor-inducible signaling components resulting in augmentation of defense response induction.
Data that illustrates the large-scale analysis of the proteome of grape, including the preparation of reference maps, comprehensive image analysis and comparisons of the properties and functions of the identified proteins will be discussed.
In conclusion, the identified proteins can help in better understanding the mechanism of induced resistance on grapevine and furnish knowledge to improve its efficacy, especially in the identification of new elicitors able to strengthening the plant's state of alert with low metabolic cost for the plant.

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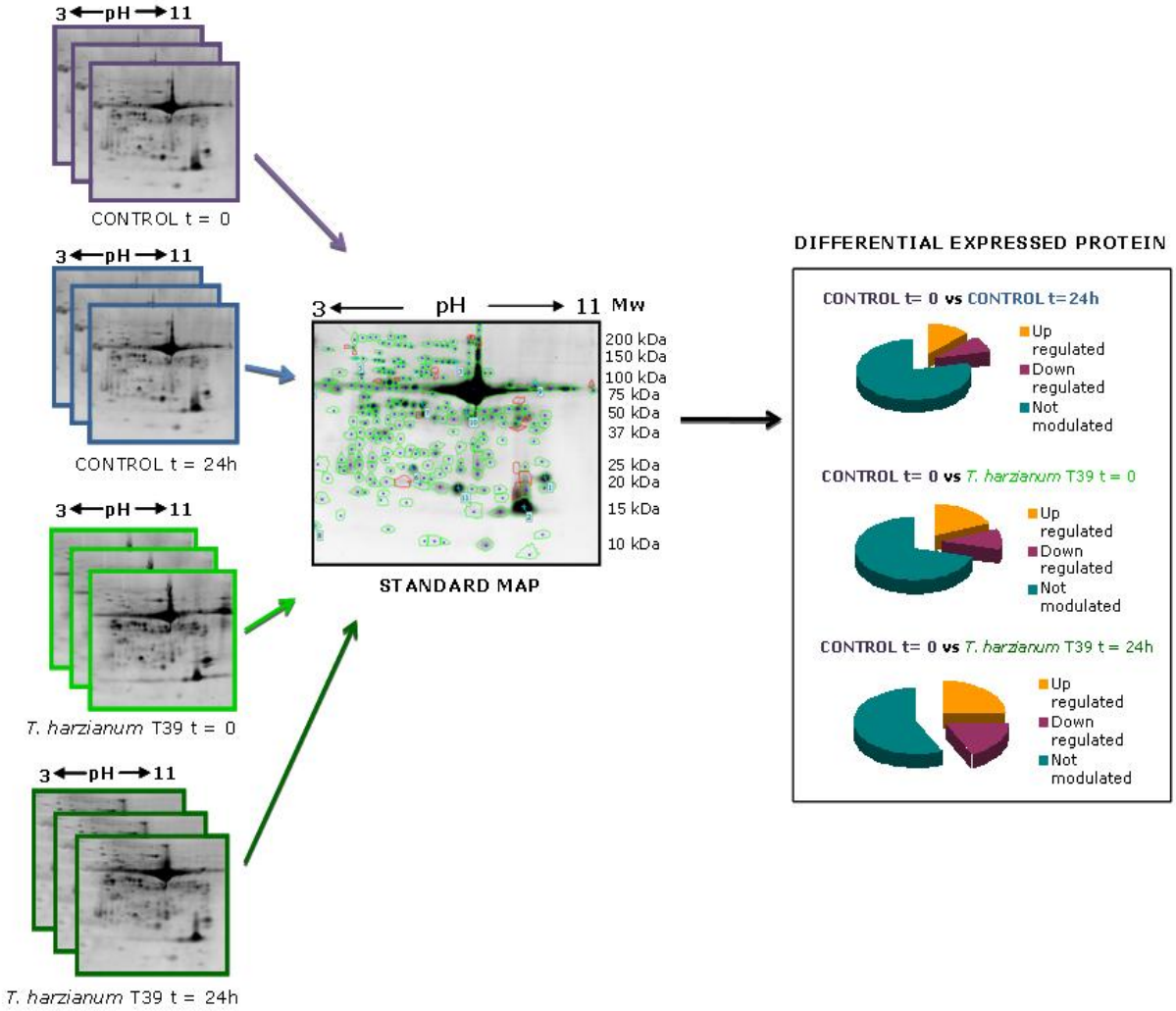

Figure 2: Identification of proteins differentially expressed in grapevine leaves treated with *Trichoderma harzianum* T39 or water (control), before and after *Plasmopara viticola.* Proteins from three independent biological replicates were separated by 2D-gel electrophoresis. 2D-maps were then analysed and compared using Image Master 2D 7.0 Software in order to create a standard map. Differentially expressed spots were then identified by MS- based technique.

Comparison of transcriptional changes associated to *Plasmopara viticola* infection in a resistant and a susceptible *Vitis* species

M. Polesani[a], L. Bortesi[a], A. Ferrarini[a], A. Zamboni[a], M. Fasoli[a], C. Zadra[b], A. Lovato[a], M. Pezzotti[a], M. Delledonne[a], A.Polverari[a]

[a]Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona, Italy, [b]Department of Agricultural and Environmental Sciences, University of Perugia, Borgo XX Giugno 74, 06100 Perugia, Italy.

Plasmopara viticola (Berk. and Curt.) Berl., the causal agent of downy mildew in grapevine, is a devastating disease with severe secondary impact on the environment due to repeated fungicide applications. *P. viticola* is an obligate pathogen that obtains nutrients from infected plant cells through haustoria, which are also thought to allow the exchange of signals involved in the establishment of compatibility. Secreted effectors of oomycetes that could suppress host cell defense responses have been described in other oomycetes, but not yet in *P. viticola* [1]. European *V. vinifera* cultivars are highly susceptible to *P. viticola*, while *Muscadinia* species and several American and Asian *Vitis* spp. exhibit varying levels of resistance [2,3]. Efforts to introgress these traits into cultivated *V. vinifera* genotypes by conventional breeding have produced some resistant interspecific hybrids, but further work is needed to couple strong resistance with high quality wine production. Detailed resistance mechanisms have been described in a few model species: plants can recognize both general and specific elicitors, as well as byproducts of pathogen activity, through a wide repertoire of receptors, with intriguing similarity to the innate immune system in animals [4]. Defense responses include strengthening cell walls, the synthesis of pathogenesis-related (PR) proteins and antimicrobial compounds such as phytoalexins and the hypersensitive response (HR) [5, 6]. It has been suggested that resistance of wild American grapevine species may depend on higher basal levels of certain antimicrobial compounds. Post-infectional resistance mechanisms have also been described in wild *Vitis* species, including the accumulation of reactive oxygen species, PR-proteins, antimicrobial compounds, peroxidase activity and the HR [7].

We analyzed the early transcriptional changes associated with *P. viticola* infection in both susceptible *Vitis vinifera* and resistant *Vitis riparia* plants, by using a Combimatrix Grapevine Microarray carrying 24,571 specific probes (http://www.combimatrix.com/tech_microarrays.htm). Our study provides the first broad overview of the molecular events underlying the early response to *P. viticola* infection in susceptible and resistant grapevine species and identifies valuable candidate genes that could be used to develop mildew-resistant commercial grapevine plants.

Results

P. viticola *developmental stages.* Leaves of *in vitro* plants were infected with *P. viticola* sporangia (30.000/ml) or treated with distilled water as a control. Samples for microscopic observation were collected at 12 and 24 hpi and stained with aniline blue (Figure 1). By 24 hpi, a delay in mycelium development was observed in *V. riparia* in comparison to *V. vinifera*. The same collection times were also chosen for microarray analysis. The experiments was repeated twice (3 biological replicates).

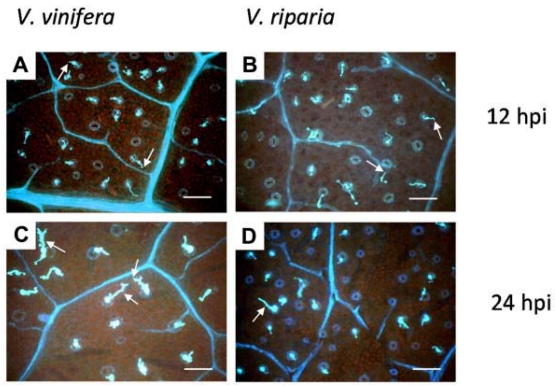

Figure 1: Early infection stages of *P. viticola* on *V. vinifera* (A and C) and on *V. riparia* (B and D) at 12 and 24 hpi.

Differences in basal gene expression among the two grapevine species. The comparison of basal gene expression levels measured by microarray analysis between healthy *V. vinifera* and *V. riparia* plants 12 and 24 h after a mock infection procedure revealed substantial variation in the expression of thousands of genes (5550 at 12 hpi and 6379 at 24 hpi), but no overall bias towards either species. Approximately 50% of those were more abundant *V. riparia* or *V. vinifera.* To test the hypothesis that resistance in *V. riparia* could depend at least in part on constitutively higher expression levels of defense genes, we selected from the lists of genes differentially expressed between the two species, the subset of all potential defense-related genes (those categorized in "resistance", "stress", "cell wall" and "secondary metabolism"). Again at both time-points, approximately half of these genes were expressed at higher levels in *V. vinifera* or in *V. riparia*. Transcript abundance averaged over whole functional categories was also similar, indicating that resistance in *V. riparia* does not seem to reflect differences in the basal expression levels of defence-related genes.

Common and species-specific transcriptional changes in V. vinifera and V. riparia in response to P. viticola infection. Figure 2 shows the total number of genes that are differentially expressed (fold change ≥2) in the two species 12 and 24 hpi. As expected, the transcriptional changes in the two species in response to infection are partially overlapping. All the common genes are modulated in the same direction by both species, indicating they probably fulfill the same functions in defense. The genes were assigned to functional categories on the basis of literature evaluation. Figure 3 shows the proportion of modulated genes whose differential expression in response to infection is observed in both species or is restricted to one or the other.

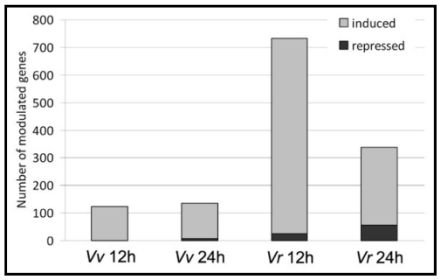

Figure 2: Total number of genes differentially expressed (fold change ≥2) in *V. vinifera* (Vv) and *V. riparia* (Vr) at 12 and 24 hpi.

The largest proportion of common genes is assigned to the "resistance" category (22%) and includes a number of genes coding for stilbene synthases and pathogenesis-related proteins, such as chitinases, 1,3glucanases and PR-10. The next largest grouping of common genes are those in the "signal transduction" category (15%), including many genes for WRKY transcription factors [8]. Approximately 12% of the common genes have metabolic functions, including a cell wall apoplastic invertase [9]. Interestingly, the strength of modulation among the common genes is invariably much higher in *V. riparia* especially for the "resistance" category. (Figure 4).

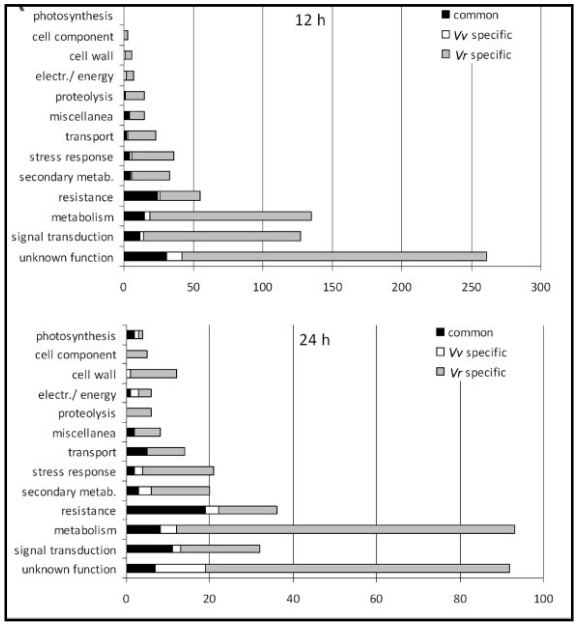

Figure 3: Proportion of modulated genes whose differential expression in response to infection is observed in both species or is restricted to one or the other at 12 (upper panel) or 24 hpi (lower panel).

In all functional categories, most of the transcriptional modulation observed in *V. riparia* is specific to that species. The most prevalent functional categories among the *V. riparia*-specific modulated genes are general metabolism and signal transduction, the latter especially at 12hpi. Transcriptional changes involving lipid metabolism include the upregulation of genes encoding enzymes involved in jasmonic acid synthesis (e.g. allene oxide synthase and cyclase, omega-3 fatty acid desaturase) [10]. In the signal transduction category, several different pathways are affected including calcium signaling [11], ethylene signaling [12], MAP kinases [13], phosphatases, receptor-like proteins and numerous transcription factors. Particularly strong modulation is observed for certain zinc-finger proteins (up to 16-fold induction) and WRKY genes (3–4-fold induction).

Whereas many "common" genes are related to general resistance mechanisms, the few resistance-related genes specifically induced in *V. riparia* are mostly associated with the hypersensitive reaction (HR). These include a homolog of the tobacco *Hin1* gene (12-fold induction) which is considered a HR marker [14]. Another HR marker, a homolog of the tomato *hsr203J* gene [15], is induced 40-fold in *V. riparia* and only 5-fold in *V. vinifera* at 12 hpi .

Full list of differentially expressed genes at:
Polesani *et al.* BMC Genomics 2010, 11:117
http://www.biomedcentral.com/1471-2164/11/117

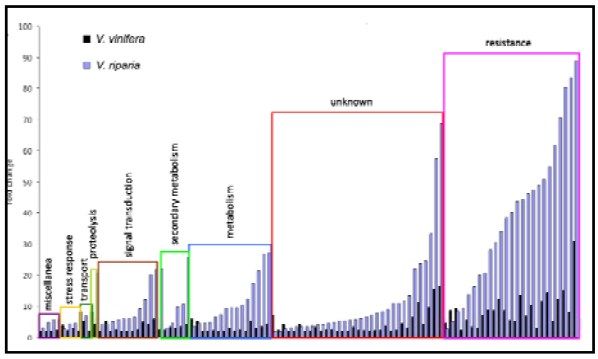

Figure 4: Comparison between fold change values recorded for all "common" genes after P. viticola infection in V. vinifera (blue) and V. riparia (light blue) at 12 hpi.

There are few genes specifically induced in *V. vinifera* at 12 hpi, but the number increases substantially by 24 hpi; these genes are scattered over different functional categories and are not particularly informative with regard to the establishment of compatible interactions.

Determination of jasmonate levels in infected leaves. The microarray data indicated that genes encoding enzymes involved in biosynthesis of jasmonic acid were strongly induced in *V. riparia* shortly after infection. We therefore measured the amount of jasmonic acid (JA) and methyl jasmonate (MeJA) [16] in the leaves of both species before infection and at four different time-points post-infection (12, 24, 48 and 96 hpi). Jasmonates were originally associated with defense against herbivores and necrotrophic pathogens but have more recently been implicated in resistance against biotrophes [17]. Our data support a role for jasmonates in the resistance of *V. riparia* against *P. viticola*, given the significant increase in the levels of both JA and MeJA at 48 hpi specifically in this species, concomitant with the effective arrest of pathogen growth, although much later in comparison to the transcriptional reprogramming described above. More experiments are needed to reveal how much of the genetic resistance response against *P. viticola* can be considered jasmonate-dependent in grapevine.

Conclusions

Our work strongly support the view that resistance in *Vitis riparia* is a post-infectional phenomenon, characterized by a rapid wave of signal transduction, followed by a re-direction of primary and secondary metabolism within 24 hpi [18]. On the opposite, early transcriptional changes observed in *V. vinifera* are mainly oriented to a weak defense response and are not informative about a possible downregulation of resistance mechanisms by pathogen effectors, which might operate later on [19]. Basal levels of defense gene expression in the two species do not seem to be responsible for the different infection outcomes. Such a wide comparative characterization of resistance and susceptible phenotypes provides several candidate genes for future functional analyses.

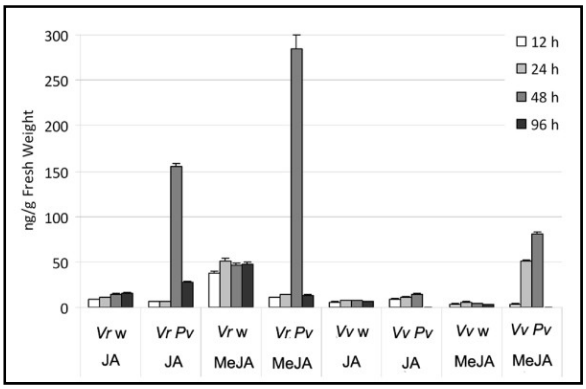

Figure 5: Jasmonic acid (JA) and methyl-jasmonate (MeJA) levels in *V. riparia* (Vr) and *V. vinifera* (Vv) at different time points after inoculation with *P. viticola* (Pv) or after mock treatment with water (w).

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Do All European cultivars have the same level of susceptibility to Downy Mildew?

M. C. Martinez, V. Alonso-Villaverde, P. Gago, J.-L. Santiago, S. Boso

Misión Biológica de Galicia, Consejo Superior de Investigaciones Científicas (CSIC), P.O. Box 28, 36080 Pontevedra, Spain. Tel: +34 986 854 800. Fax: +34 986 841 362. Correspondence to carmenmartinez@mbg.cesga.es

Grapevine Downy mildew caused by the Oomycete *Plasmopara viticola* (Berk. et Curt.) Berl. et de Toni, is one of the most important diseases from the economic point of view in the grape producing areas with rainy springs/summers as some from Spain. Fungicides are the most important control technique on susceptible cultivars grown in these areas with high disease pressure. The pathogen has been introduced to Europe in the last quarter of the 19th century and in 1878 the first symptoms were observed in the Bordeaux area. In the following decade all classical European grapevine cultivars showed to be highly susceptible, resulting in a severe pandemic throughout the continent (Millardet, 1883, Viala, 1893, Ravaz, 1911). The cellular and molecular mechanism by which the *P. viticola* infects grapevine is unknown. A number of studies deal to characterize the defense reactions in tolerant or resistant grapevines (Derks and Creasy, 1989; Kortekamp *et al.*, 1998; Gindro *et al.*, 2003) or in response to inducers of defense reactions (Hamiduzzaman *et al.*, 2005). Observations in the field indicate differences in the susceptibility to *P. viticola* among the European grapevine cultivars. Different procedures have been developed: field observations in natural conditions of infection, glasshouse-based and laboratory-based screening methods using artificial inoculation with *P. viticola* (Liu *et al.*, 2003; Boso *et al.*, 2004, 2005). In laboratory-based techniques, several types of explants have been used: detached leaves (Song *et al.*, 1998), leafed single-node-cuttings (Liu *et al.*, 2003 and 2008) leaf discs (Staudt and Kassemeyer, 1995; Brown *et al.*, 1999a; Boso *et al.*, 2006; Sotolar, 2007; Boso and Kassemeyer, 2008) or *in vitro* plantlets (Barlass *et al.*, 1986). Leaf discs tests are widely used because they are space-saving and reproduce rather accurately field responses in plants (Brown *et al.* 1999a; Sotolar, 2007). However, up to date, only few studies comparing the susceptibility of the classical European *V. vinifera* cultivars have been undertaken (Kortekamp *et al.*, 2003; Boso *et al.*, 2007). The questions, "do all European cultivars have the same level of susceptibility or are there distinct differences among them?" are still topical and of great applied and scientific importance.

Therefore, the aim of the present study was to characterize the level of susceptibility to *P. viticola* in European grapevine cultivars with field and greenhouse-grown plants as well as laboratory-based techniques.

Material and Methods

Cuttings of 13 *V. vinifera* cultivars, common in European viticultural regions (Albariño, Caiño Tinto, Caiño Blanco, Mencía, Cabernet Sauvignon, Alicante Bouschet, Treixadura, Torrontés, Godello, Blanco Lexítimo, Castañal, Albarello, Chasselas) and Jacquez and two rootstocks (110-R, SO4), were cultivated under greenhouse conditions. The European cultivars of *V. vinifera* represented susceptible genotypes, whereas the Jacquez and the rootstocks are of resistance model (partially and resistant respectively). A population of *Plasmopara viticola* was obtained from naturally infected plants in the vineyards around Galicia (Spain) and maintained on *V. vinifera* Albariño, grown in the greenhouse. Sporangia were raised in order to prepare an inoculum following the method of Rumbolz *et al.* (2002). For this purpose, the adaxial leaf surfaces of the plants were sprayed with a suspension of 40,000 sporangia·ml^{-1} in distilled water and covered overnight with a wet polythene bag. The inoculated plants were kept under high relative humidity (RH) (> 96 %) overnight at 24 °C. After incubation for 5 to 6 days (dpi) under greenhouse conditions, the plants were again maintained under moist conditions overnight to induce sporulation. From the sporulating lesions, freshly developed sporangia were collected in centrifuge tubes using a small paintbrush. The inoculum was prepared by counting these sporangia using a Fuchs- Rosenthal chamber and adjusting to a dilution of 25,000 sporangia·ml^{-1}.

This research was conducted to field (Boso *et al.*, 2005), in laboratory (assays were performed with leaf discs) and in greenhouse-grown plants. Plants were inoculated with *P. viticola* in the laboratory using the leaf disc (Staudt and Kassemeyer, 1995; Rumbolz *et al.*, 2002) and plant (Boso *et al.*, 2008) techniques. The results obtained with both methods were compared. Disease severity (sporulation, necrosis and oil spots), disease incidence (sporulation, necrosis and oil spots) and sporulation density (see above) were analyzed. The disease incidence was calculated as the number of leaves with sporulating lesions at the abaxial surface (disease incidence of sporulation), with necrosis (disease incidence of necrosis), or oil spots (disease incidence of oil spots) per total number of leaves per plant. To score disease severity we estimated the percentage of the leaf area exhibiting symptoms of sporulation (disease severity of sporulation), with necrosis (disease severity of necrosis), or oil spots (disease severity of oil spots). All experiments were performed in triplicate.
Each variant was examined by analysis of variance (ANOVA) to determine significant differences between varieties. For each variant, Fisher's protected test (minimum significant difference [LSD] method) (Steel *et al.*, 1997) was used to determine the level of resistance or susceptibility, for each cultivar. All calculations were performed using SAS V8.1 software (SAS 2000).

Results and Discussion

The statistical analysis in field and in our inoculation experiments revealed a different level of susceptibility to *P. viticola* among the *Vitis vinifera* cultivars (P=0.001). As expected, 110-Ritcher and SO4 were separated from the studied varieties in all experiments, as these rootstocks were not very affected by *P. viticola.*

Field (Figure 1). Treixadura and Caiño Blanco were the most susceptible cultivars, while Castañal, Cabernet Sauvignon and Albarello showed less susceptibility.

Laboratory (discs test). The results obtained demonstrated, as it was expected, all viniferas to present 100% incidence of sporulation, 0% incidence of necrosis and 0% severity of necrosis (Figure 2). Regarding to the rootstocks, the incidence of sporulation was 84% for 110-Ritcher and only 5% for SO4. The incidence of necrosis ranged from 86% in 110-Ritcher to 90% in SO4 while the severity of necrosis was the same for both rootstocks. It is important to highlight the different shape of the necrosis observed in these rootstocks. As reported in previous works (Boso *et al.*, 2006, 2008), slightly variability was observed within the viniferas taken into account the severity and the density of sporulation. Regarding to the first parameter, three groups were clearly distinguished: The first group was formed by Treixadura, Albariño, Caiño Blanco and Caiño tinto, with the highest severity of sporulation. The second one grouped Castañal and SO4 with the lowest percentage (8% in SO4) and finally, the rest of varieties, with a medium percentage, formed a third group.

Significant differences were also observed in relation to the density of sporulation. Treixadura, Albariño, Alicante and Caiño Blanco showed the high density while Castañal, Cabernet Sauvignon, Chaselas and rootstock SO4 presented the lowest values for this parameter.

Greenhouse-grown plants. By one hand, Torrontés, Alicante, Godello, Albarello, Treixadura and Albariño showed the highest incidence and severity of sporulation as well as oil spots. By the other hand, Castañal, Chasselas and Jacquez, beside the two rootstocks, presented the lowest values. The incidence and severity of necrosis could only be detected in the rootstock SO4 as no necrosis were observed in the remaining Vitaceae.

Not all European viniferas present the same level of susceptibility to downy mildew. This work has allowed us to differentiate at least two groups: Highly susceptible varieties (Treixadura, Caiño Blanco and Albariño), little susceptible (Castañal and Albarello). However, this assumption has to be further investigated by the way of quantitative analyses on the resistance response in the studied genotypes.

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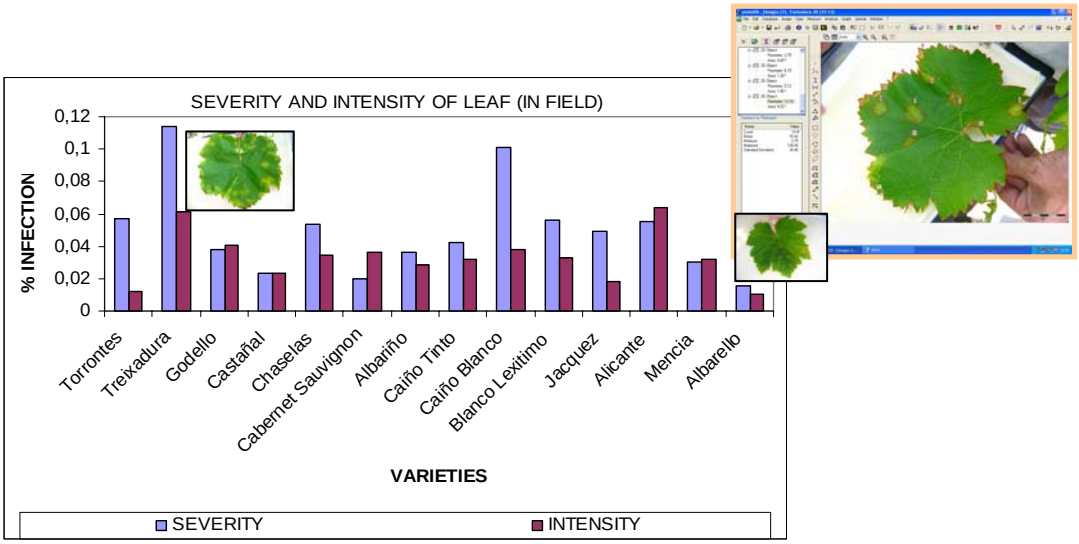

Figure 1: Disease severity and Intensity sporulation of leaves in field

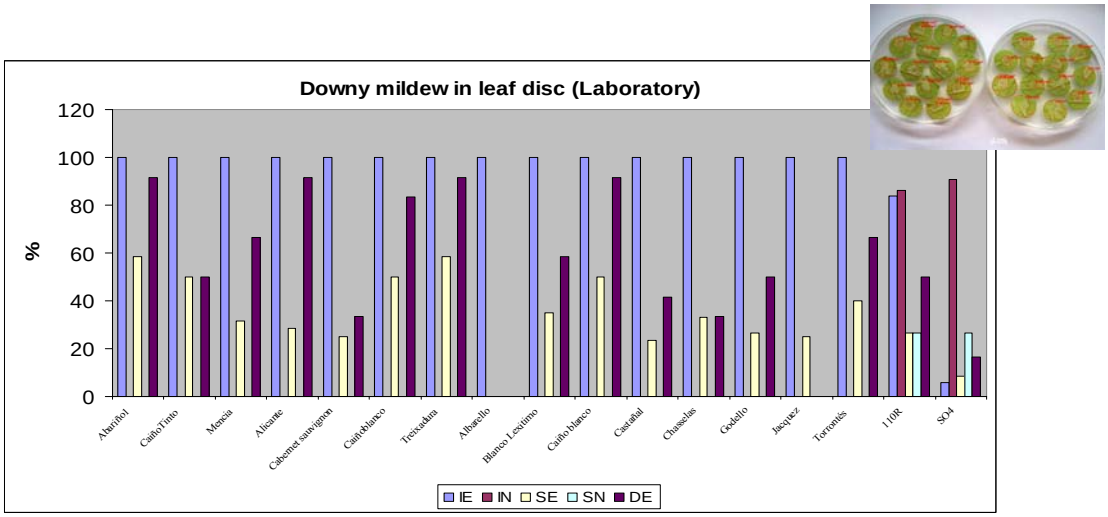

Figure 2: Different parameter for downy mildew measured in laboratory (assays were performed with leaf discs). IE: Disease incidence of sporulation, IN: Disease incidence of necrosis, SE: disease severity of sporulation, SN: disease severity of necrosis, DE: Density of sporulation.

Effect of transient expression of two grapevine chitinases upon infection by *Plasmopara viticola* and *Erysiphe necator*

A.S. Miclot, M.A. Dorne, D. Merdinoglu, P. Mestre

UMR 1131 INRA / Université de Strasbourg Santé de la Vigne et Qualité du Vin, 28 rue de Herrlisheim, BP 507, 68021 Colmar Cedex, France

Powdery mildew caused by the Ascomycete *Erysiphe necator* and downy mildew caused by the Oomycete *Plasmopara viticola* are two of the most important grapevine diseases worldwide. These obligate biotrophs can drastically reduce yield and quality of grapes leading to important economic losses. Control of these pathogens is mainly based on the use of fungicides. These chemical treatments can cause damage to the environment and increase production costs considerably. Thus, there is a need for alternative strategies to reduce these treatments. An alternative, cost-effective and environmentally friendly strategy is the use of varieties showing resistance to the pathogens.

Accordingly, at INRA Colmar we are developing a breeding program for resistance to grapevine downy and powdery mildew. The program exploits mainly the resistance to *P. viticola* and *E. necator* found in *Muscadinia rotundifolia*, whereas other sources of resistance are being characterised. The analysis of the genetic basis of the resistance from *M. rotundifolia* resulted in the identification of two resistance genes against downy mildew, named *Rpv1* and *Rpv2*, and one resistance gene against powdery mildew, named *Run1*. Based on the position of these genes on the grapevine genetic map, they are members of the NBS-LRR class of canonical plant disease resistance genes.

Genes for resistance to fungal diseases are of special interest for improving grapevine cultivars, so it is important to have efficient tools to study their function. Stable genetic transformation of grapevines is today performed with both *Agrobacterium*-mediated and biolistic systems, but it is time consuming and shows low efficiency. In order to evaluate gene function in grapevine leaves, a method of transient expression of genes using *Agrobacterium* was developed at our laboratory (Santos-Rosa *et al.*, 2008). While this method allowed the transient expression of marker genes, it remains to be confirmed as suitable to analyse the function of plant disease resistance genes.

Since no NBS-LRR genes from grapevine have been cloned to date, we used other genes to test the suitability of our method for the analysis of disease resistance. Genes encoding hydrolytic enzymes such as chitinases, which can degrade fungal cell wall components, are attractive candidates for improving disease resistance. As a matter of fact, it has been shown that the introduction of rice chitinase RCC2 into grapevine enhances resistance against powdery mildew (Yamamoto *et al.*, 1999). Thus, we searched the grapevine genome sequence for chitinases highly similar to RCC2 and found two chitinases showing 59% and 58% identity at the amino acid level, which we called respectively Chi22 and Chi88. Both chitinases were cloned from cDNA and genomic DNA from the *Vitis vinifera* line 40024 into binary vector pBIN 61 to be used in transient expression experiments. The identity of the cloned genes was confirmed by sequencing.

Evaluation of the effect of Chi22 and Chi88 expression on pathogen infection will be done by infiltrating *Agrobacterium* containing those genes into grapevine leaves and challenging the infiltrated leaves with the pathogens. *Agrobacterium* suspensions will be performed as described (Santos-Rosa *et al.*, 2008). The first and second fully expanded leaves from *in vitro* grown plants of *Vitis vinifera* cultivar Syrah and *Vitis vinifera* line 40024 will be detached and submerged abaxial face down in an *Agrobacterium* suspension containing the chitinase constructs to be vacuum-infiltrated. The leaves will be rinsed in sterile water and placed on sterile water-soaked filter paper in a sealed 90 mm Petri dish. Gene expression is expected within 3 days. Thus, 3 days after infiltration, leaves will be inoculated with *P. viticola* or *E. necator*. The Petri dishes will be incubated in a growth chamber at 21°C. Non-infiltrated leaves, or leaves infiltrated with an empty vector, will be used as controls. Susceptibility to powdery or downy mildew infection will be evaluated after inoculation, based on a disease index. The number of spores produced will be assessed using a Beckmann-Coulter cell counter.

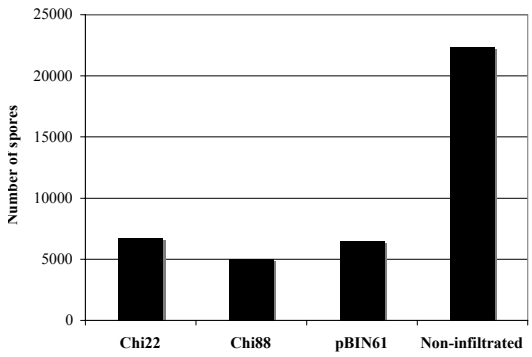

Figure 1: Quantification of powdery mildew infection in leaves, expressed as number of spores produced 11 days after inoculation with *E. necator*. Data shown represent the total spore number of seven leaves.

Preliminary results for inoculation with *E. necator* showed that the number of spores produced on non-infiltrated leaves was more than 3 times higher than on infiltrated leaves. However, we did not find differences between the number of spores produced on leaves infiltrated with chitinases or with pBIN61 (Figure 1). Experiments aiming to confirm these results as well as to study the effect of the expression of chitinases on downy mildew infection are on course.

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The "oil spot" in *P. viticola* infected grapevine leaves: a site of source-sink transition?

M. Gamm, MC. Héloir, P. Frettinger, D. Wendehenne, M. Adrian

Unité Mixte de Recherche INRA 1088/CNRS 5184/ Université de Bourgogne Plante-Microbe-Environnement, 17 rue Sully, BP 86510, 21065 Dijon cedex, France

The colonization of a plant with a biotrophic pathogen often results in an extensive re-programming of the host's metabolism. Changes of sugar repartition and photosynthetic capacity are often described in the context of a source-sink transition, mostly in connection to an increase in an invertase activity (Roitsch, 1999; Walters & McRoberts, 2007). This sucrose-cleaving enzyme can be of host or pathogen origin and is typically responsible for an accumulation of hexoses at infection sites (Roitsch & González, 2004).

In grapevine leaves infected by *Plasmopara viticola*, we showed, by chlorophyll fluorescence measurements, a decrease of photosynthetic capacity over the course of infection. Furthermore, infected areas displayed an abnormal presence of starch at the end of the night period. This observation suggests an infection-induced metabolic deregulation which could be the origin or the outcome of a source-sink transition.

In order to better characterize the observed phenomenon, soluble sugars were quantified in infected and healthy tissues over a time-course from 0 to 7 days post infection (dpi). In *P. viticola* infected leaves, an accumulation of the hexoses glucose and fructose was observed from 5 dpi, beginning after the apparition of symptoms in our conditions. To analyze whether the accumulation of these sugars could be linked to a changed invertase activity, the enzyme was quantified in healthy and infected grapevine leaves. Different pH and fractions of protein extraction were assessed. In the fraction containing insoluble enzymes, at pH 7.5, a strong increase of invertase activity was measured, starting from the apparition of symptoms.

In parallel, the gene expression analyses of a grapevine cell wall invertase and a *P. viticola* isoform revealed the induction of these genes during infection.

Taken together, these results suggest a source-sink transition in *P. viticola* infected grapevine leaves during the development of the pathogen as described for other plant interactions with biotrophic pathogens (Walters & McRoberts, 2007).

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Evaluation of reference genes for gene expression normalization in *V. vinifera* cv. Marselan by quantitative real-time RT-PCR

M. Gamm, MC. Héloir, J. Kelloniemi, B. Poinssot, D. Wendehenne, M. Adrian

Unité Mixte de Recherche INRA 1088/CNRS 5184/ Université de Bourgogne Plante-Microbe-Environnement, 17 rue Sully, BP 86510, 21065 Dijon cedex, France

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Grapevine (*Vitis vinifera* cvs.) gene expression data are crucial for better understanding of the plant's interactions with its numerous pathogens. Quantitative real-time PCR is an important tool to produce reliable and sensitive information on gene expression changes, but appropriate analysis of the measured expression data requires the use of an internal control to allow the comparison of different samples. Reference genes that are stably expressed in all samples are commonly used for normalization. However, the expression stability of a candidate reference gene has to be validated, as confirmed by recent studies describing the variation of typical housekeeping genes and its dependence on the experimental setup (Guenin *et al.,* 2009).

Here, we describe the identification of reference genes suitable for qRT-PCR studies of *Vitis vinifera* cv. Marselan leaves infected by *Plasmopara viticola* and berries infected by *Botrytis cinerea*. Among the 12 candidate reference genes analyzed, 9 were selected on the basis of a microarray expression profile and three commonly used internal control genes (18S rRNA, Actin, EF1-α) were included in the analysis. The expression data obtained with the validated primer pairs was analyzed by three different complementary statistical approaches.

geNorm (Vandesompele *et al.,* 2002), *Normfinder* (Andersen *et al.,* 2004) and *BestKeeper* (Pfaffl *et al.,* 2004) use different statistical algorithms to assess the expression stability of every gene in the studied data set. The comparison of the results suggested that, for the studied conditions, the 18S rRNA and the Actin gene, often used as grapevine housekeeping genes, are the least stable of the analyzed candidates. While some differences were visible between the berry and leaf data sets, a number of genes were expressed at a constant level in both leaves and berries, infected or not. The genes with the most stable expression in the studied conditions were ATP6V0C and 60SRP. Additionally, UBE3 and SMD3 could be identified as suitable reference genes but only for berry and leaf samples, respectively.

These genes constitute useful candidates for reference gene selection for further studies on grapevine gene expression.

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Marker-based selection for powdery mildew resistance genes in different grape hybrid families

D. Katula-Debreceni[a], A. Veres[a], A. Szőke[a], AK. Lencsés[a], P. Kozma[b], S. Hoffmann[b], E. Kiss[a]

[a]Szent István University, Institute of Genetics and Biotechnology, Páter K. u. 1. H-2100 Gödöllő, Hungary
[b]Research Institute of Viticulture and Enology, Pázmány P. u. 4. H-7634 Pécs, Hungary

Incorporating durable resistance to the most important fungal diseases (powdery and downy mildew, PM and DM) into cultivars with excellent grape quality has become a crucial objective of European grapevine breeding since the second half of the 19th century when these pathogens were imported from North-America into Europe. Different resistance (*R*) genes appear to detect the pathogen by different mechanisms. Therefore, resistance conferred by a combination of various *R* genes is more difficult for the pathogen to overcome than resistance due to a single *R* gene (McDonald & Linde, 2002). Introgression of resistance genes from wild *Vitis* species is one of the possible ways of achieving resistance or increased tolerance in susceptible *Vitis vinifera* L. cultivars. Since no *V. vinifera* cultivars carrying major PM resistance genes were found until the mid 1960s, wild *Vitis* species were used as sources of resistance genes. Because of their low quality, many crosses to *V. vinifera* were required to recover the high quality of *vinifera* cultivars (Fischer *et al*., 2004). In Hungary, grapevine resistance breeding started in 1949 with the aim of producing PM and DM resistant cultivars. For this purpose French-American hybrids were used (Kozma, 1999). Crossing of the mildew resistant Villard blanc variety resulted in cultivars with resistance and high quality, e.g. Zalagyöngye, Bianca, Medina, Nero (Csizmazia & Bereznai, 1968). However, many wild *Vitis* species carry high or partial resistance to the pathogens. *Muscadinia rotundifolia* MICH. SMALL was described as totally resistant to PM and DM (Boubals, 1961). Because of this it was used in breeding programs in Europe (Merdinoglu *et al*., 2003), including Hungary (Kozma & Dula, 2003). The Research Institute of Viticulture and Enology in Pécs pioneered combining resistance genes derived from different sources. A BC_4 hybrid (*M. rotundifolia* L. x *V. vinifera*, VHR 3082-1-42) produced by Bouquet (1986) was brought into Hungary in 1996 and was crossed with different *V. vinifera* cultivars and complex hybrids carrying oligogenic mildew resistance genes. However, while *V. vinifera* cultivars are classified as susceptible, different cultivars show varying levels of susceptibility (Boubals, 1961). Filippenko & Stin (1977) identified a PM resistant cultivar, 'Dzhandzhal kara' and used it in the breeding programs. Later additional resistant cultivars from Central Asia were identified (Coleman *et al*., 2009). These cultivars could be very valuable sources of resistance genes in breeding programs, because contrary to wild *Vitis* species, they do not have an undesirable impact on grape quality. Beside 'Dzhandzhal kara', whose resistance was also used in Hungary (Korbuly, 1999), 'Kishmish vatkana' is another potential source of PM resistance (Kozma *et al*., 2006, Coleman *et al*., 2009).

The genetic basis of resistance of *M. rotundifolia* deciphered by Donald *et al*., 2002 was the gene was named *RUN1* (Resistance to *Uncinula necator*). Thanks to molecular marker mapping, the *RUN1* locus was localised in linkage group (LG) 12 (Barker *et al*., 2005). In *M. rotundifolia,* a resistance gene against DM, *RPV1* (Resistance to *Plasmopara viticola*), was also identified in the same LG (Wiedemann-Merdinoglu *et al*., 2006, Dry *et al*., 2010). Segregation analysis revealed that the candidate resistance gene of *V. vinifera* origin was inherited in a Mendelian manner. A 1:1 phenotypic segregation ratio was obtained in the progeny of a Nimrang x Kishmish vatkana cross (Kozma *et al*., 2006) showing that a single dominant gene, *REN1* (Resistance to *Erysiphe necator*), is responsible for the resistance (Kozma *et al*., 2006). Molecular analysis of the progeny proved that the *REN1* gene differs from the *RUN1* since it has been localized in LG 13 (Hoffmann *et al*., 2008).

Molecular marker-assisted selection (MAS) facilitates the precise identification of seedlings that have inherited the desired gene, even before the expression of the trait is observable in the progeny. Large numbers of DNA sequence-based markers have been developed in grapevine which, in turn, made the construction of genetic linkage maps possible (Doligez *et al*., 2006, Di Gaspero *et al*., 2007). Recent publication of the *V. vinifera* genome sequence (Jaillon *et al*., 2007) is accelerating the development of new SSR (Simple sequence repeat) markers and is allowing them to be anchored to physical maps. Codominant SSR markers are particularly useful, when multiple genes that encode the same phenotype are to be introgressed into a single genome, because they enable breeders to simultaneously select for several genes in a progeny.

There are several reports about the application of DM and PM resistance gene linked markers for selection. Inheritance of *RUN1* was followed in a VHR 3082-1-42 x Regent hybrid by Eibach *et al*. (2007) and a VHR 3082-1-42 x Cardinal cross by Molnár *et al*. (2007). *REN1*-linked markers were determined in a Nimrang x Kishmish vatkana progeny (Hoffmann *et al*., 2008). QTL markers found in the cultivar Regent, efficient against PM and DM and belonging to LG 15 and LG 18 (Akkurt *et al*., 2007), were used by Eibach *et al*. (2007).

Different hybrid families were produced by Kozma *et al*., 2006 to combine PM and DM resistance genes. We analysed four from these families: BC_4 (VRH 3082-1-42) x *V. vinifera* cv. Kishmish vatkana, BC_4 x *V. vinifera* cv. Kishmish moldavskij, *V. vinifera* cv. Génuai zamatos x *V. vinifera* cv. Kishmish vatkana, (Laszta x *V. vinifera* cv. Dzhandzhal kara) x (*V. vinifera* cv. Katta kurgán x *V. vinifera* cv. Perlette). The purpose of our study was to select with the linked markers the genotypes carrying pyramided resistance genes and to follow the single resistance genes in the progenies. The plants carrying pyramided resistance genes for the same phenotypes can be identified only with DNA analyses. With the testing of the progeny of the (Dzhandzhal kara x Laszta) x (Katta kurgán x Perlette) cross, containing a dominant PM gene from Dzhandzhal kara and QTLs from Laszta, we wanted

to check the applicability of the *RUN1/RPV1* or *REN1* linked markers for following the PM resistance gene from Dzhandzhal kara. Since among the parents of Laszta, SV20365 and SV12375 clones of Seyve Villard origin are present, the plants were also tested with PM-linked QTL markers.

DNA isolation and marker analysis. Young leaves of the hybrid individuals and parent grapevines (Table 1) were collected in 2007 and 2008 and stored at -20ºC until DNA isolation. Genomic DNA was isolated using a DNeasy Plant Mini Kit (Qiagen, 2006). PCR was performed in a BioRad iCycler as described by Galbács *et al*. (2009). To determine the exact size of PCR amplicons, they were fractionated in an 8% polyacrylamide gel (ReproGel™, GE Healthcare, AP Hungary Kft) in a vertical system (ALF-Express II., Amersham Biosciences, AP Hungary Kft, Budapest) using Cy-5 fluorescent labelling.

Results

SSR results of the resistance gene linked markers are presented in Tables 3-6. In the BC_4 x Kishmish vatkana hybrid family, the BC_4 parent is heterozygous for the *M. rotundifolia RUN1*/*RPV1* genes. Therefore the alleles 160 bp, 294 bp or 122-122 genotype (with markers VMC8g9, VVim11, VMC1g3.2, respectively) indicate the presence of the resistance genes. In Kishmish vatkana, which is heterozygous for the *REN1* locus, genotypes of 260-260 and alleles 286 bp, 164 bp are the markers for PM resistance (with markers VMCNg4e10.1, VMC9h4.2 and UDV20, respectively). Among 441 symptomless individuals all the expected genotypes, *RUN1*/*RPV1*/*REN1, RUN*/*RPV1*, *REN1,* could be identified.

Table 1. Hybrid families and parent grape varieties analysed

	Number of analysed plants in progeny	
Variety	PM symptomless*	PM susceptible*
BC_4 / VRH 3082-1-42	yes	no
Kishmish vatkana	yes	no
Kishmish moldavskij	no	yes
Génuai zamatos	no	yes
Dzhandzhal kara	yes	no
Laszta	yes	no
Regent	yes	no
Dzsandzsal kara x Laszta	yes	no
Katta kurgán x Perlette	no	yes
BC_4 x Kishmish vatkana	441	30
BC_4 x Kishmish moldavszkij	30	20
Génuai zamatos x Kishmish vatkana	78	68
(Dzsandzsal kara x Laszta) x (Katta kurgán x Perlette	96	30
BC_4 x Kishmish vatkana	441	30

* PM symptoms on leaves were phenotypically evaluated at the Research Institute of Viticulture and Enology, Pécs.

In the cross of BC_4 x Kishmish moldavskij, Kishmish moldavskij is sensitive to PM and DM. Therefore the progeny carries only the *RUN1*/*RPV1* dominant genes of BC_4. Consequently, the resistance linked marker alleles or genotypes are as follows: 160-174 bp, 160-160; 294-294; 122. The resistant progeny of Génuai zamatos x Kishmish vatkana is supposed to possess the 164 bp *REN1* linked marker alleles with UDV20 (Table 5) derived from the Kishmish vatkana parent, since Génuai zamatos is a PM susceptible *V. vinifera* cultivar. Results concerning (Dzhandzhal kara x Laszta) x (Katta kurgán x Perlette) compiled in Table 6 prove that only *REN1* linked marker alleles cosegregate with the PM symptomless phenotype, suggesting that Dzhandzhal kara has the same PM resistance gene as Kishmish vatkana. Susceptible and symptomless progenies cannot be differentiated with *RUN1*/*RPV1* linked markers, showing that the hybrid population does not carry these genes. QTL analyses with the primers listed in Table 2 are in progress.

Discussion

Markers applied for selecting both individual and pyramided resistance genes proved to be applicable MAS in all four hybrid families. Our data also corroborated earlier findings that the *M. rotundifolia*-derived *RUN1* and *RPV1* loci are linked. Comparing the symptomless progenies derived from the crosses BC_4 x Kishmish vatkana and (Laszta x Dzhandzhal kara) x (Katta kurgán x Perlette) using resistance gene (*RUN1*/*RPV1* and *REN1*) linked markers proved that these two Central Asian cultivars presumably contain identical PM resistance genes, confirmed by Coleman *et al*. (2009).

Table 2. Molecular markers applied in the analyses

Markers		References
REN1	UDV020	Di Gaspero *et al*. 2007 Hoffmann *et al*. 2008
	VMC9h4.2	
	VMCNg4e10.1	
RUN1/ RPV1	VMC8g9	Doligez *et al*. 2006
	VVim11	Doligez *et al*. 2006
	VMC1g3.2	Merdinoglu *et al*. 2003
PM QTL	ScORA7-760 VMC4d9.2, UDV15b, VViV67	Akkurt *et al*. 2007, Eibach *et al*. 2007
DM QTL	VMCNG2f12, UDV130, UDV108	Eibach *et al*. 2007

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Table 3: SSR marker results in the BC_4 x Kishmish vatkana hybrid family

Variety	*RUN1/RPV1*			*REN1*		
	VMC8g9	VVIm11	VMC1g3.2	VMCNg4e10.1	VMC9h4.2	UDV20
BC_4	**160**-167	272-**294**	**122**-142	260-260	282-298	148-148
Kishmish vatkana	167-174	260-284	122-142	240-**260**	262-**286**	138-**164**
Susceptible progeny	167-167 167-174	272-284 260-272	122-142 142-142	240-260	262-282 262-298	138-148
Symptomless progeny	**160**-167 **160**-174	260-**294** 284-**294**	122-142 **122**-122	**260**-260	282-**286** **286**-298	148-**164**

Table 4: SSR Marker results in the BC_4 Kishmish moldavskij family

Variety	*RUN1/RPV1*		
	VMC8g9	VVIm11	VMC1g3.2
BC_4	**160**-167	272-**294**	**122**-142
Kishmish moldavskij	160-174	294-294	128-142
Susceptible progeny	160-167 167-174	272-294	142-142 128-142
Symptomless progeny	**160**-174 **160**-160	294-**294**	**122**-142 **122**-128

Table 6: SSR marker results of (Dzsandzsal kara x Laszta x) x (Katta kurgán x Perlette) progeny progeny

Variety	*RUN1*		*REN1*		
	VMC8g9	VMC1g3.2	VMCNg4e10.1	VMC9h4.2	UDV20
Kishmish vatkana	167-174	122-142	240-**260**	262-**286**	138-**164**
Dzhandzhal kara	167-174	124-128	255-**260**	280-**286**	150-**164**
Laszta	162-178	128-134	230-268	252-290	150-150
Dzsandzsal kara x Laszta	162-174	124-128	**260**-268	**286**-290	150-**164**
Katta kurgán x Perlette	178-178	122-128	238-260	262-286	138-150
Susceptible progeny	162-178 174-178	128-128 122-124 124-128	238-268 260-268	262-290 286-290	138-150 150-150
Symptomless progeny	162-168 174-178	124-128 122-128 122-124 128-128	238-**260** 260-**260**	262-**286** 286-**286**	138-164 150-**164**

Table 5: SSR marker results of Génuai zamatos x Kishmish vatkana

Variety	*REN1*
	UDV20
Génuai zamatos	138-148
Kishmish vatkana	138-**164**
Susceptible progeny	138-138 138-148
Symptomless progeny	138-**164** 148-**164**

*Shaded numbers mark alleles linked to resistance

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Session 2: Biology of the pathogens, Ontogenic resistance, pathogen impact on plant physiology, population genetics

Acute Low Temperature Events Reduce the Survival of *Erysiphe necator* and Increase Resistance in Ordinarily-Susceptible *Vitis vinifera* Leaf Tissue

M.M. Moyer[a], D.M. Gadoury[a], L. Cadle-Davidson[a,b], I.B. Dry[a,c], P.A. Magarey [d], W.F. Wilcox[a], R.C. Seem[a]

[a]Department of Plant Pathology and Plant-Microbe Biology, Cornell University and [b]USDA-ARS, Grape Genetics Research Unit, New York State Agricultural Experiment Station, 630 West North Street, Geneva, New York, 14456, USA; and [c] CSIRO Plant Industry, Glen Osmond, South Australia, 5064, Australia; [d]Magarey Plant Pathology, PO Box 220, Loxton, South Australia 5333

Introduction

The effect of temperature on the development of *Erysiphe necator*, syn. *Uncinula necator*, Schwn. Burr) has been the subject of several research reports over the last 60 years (Austin *et al.*, 2009; Chellemi *et al.*, 1991; Delp, 1954; Gadoury and Perason, 1988-1990; Hill, 1990; Jailloux *et al.*, 1998; Schnathorst, 1965; Ypema and Gubler, 1997). Much of this research focused on defining the optimal temperature range (Delp, 1954) or the lethality of high temperatures (*i.e.* >32°C) on powdery mildew growth and development (Austin *et al.*, 2009; Chellemi *et al.*, 1991; Delp, 1954). However, in cooler viticultural regions (e.g., northeastern North America, the Mosel region of Germany, or Tasmania, Australia) temperatures are rarely high enough to impact survival of mildew colonies, and temperatures during the growing season remain within the optimal ranges for extended periods. Despite this apparent favorability, the pre-bloom increase of foliar incidence is unusually slow. Even within high-inoculum vineyards, the first mildew colonies are rarely detected until 4 to 5 weeks after budbreak. Interestingly, a similar phenomenon is also seen in South Australia (*P. Magarey, personal communication*). While these two locations have little in common with respect to daily temperatures after bloom, both experience relatively cold nighttime temperatures (e.g., > 4°C) in the period before bloom. From these observations, we hypothesized that cold temperatures either increased host resistance via abiotic stress responses, and/or negatively impacted existing powdery mildew colonies.

Impact of pre-inoculation exposure of the host to low temperature upon resistance to infection. Abiotic stresses on a plant may affect the development of obligate biotrophs due to the nature of their highly coordinated development and parasitism. To test whether low temperatures induced resistance in normally susceptible tissue, we detached, highly susceptible seedling leaves from *V. vinifera* cv. 'Chardonnay', exposed them to 2, 4, 6, or 8°C for 2, 4, 6, or 8 h, and inoculated with a conidial suspension 24 h later. Colonies were allowed to develop for 5 days thereafter at 22°C. Colony area, as computed from 2 transects of the colony, was recorded. This allowed us to assess "longer" term effects of low temperature (LT) pretreatment on colony development.

In a second series of experiments, the LT treatments were 2, 4, 6, or 8°C for either 2 or 8 h. Control treatments remained at 22°C. At 24 h post-LT exposure, leaves were dusted with conidia, incubated at 22°C for 48 h, and were then fixed in a 3:1 ethanol:acetic acid solution before being stained with Commassie Brilliant Blue. The first 100 conidia were then counted and classified as (i) germinated, (ii) primary hypha, or (iii) branched hyphae development. This assay helped to determine the shorter-term effects of LT pre-inoculation treatments on infection and early establishment.

Results

Pre-inoculation LT treatment significantly reduced overall colony size from 37 to 55% compared to controls. Interestingly, the degree (temperature) of the LT treatment no significant effect (Tukey's HSD, significance at $P<0.05$), nor the duration of the LT event within a given temperature (slope coefficient not significant at $P=0.41$). However, all were significantly less than the control, indicating that while the degree and duration of a cold event may not be quantitative, the occurrence of a low temperature event has a qualitative effect on subsequent mildew development (Figure 1).

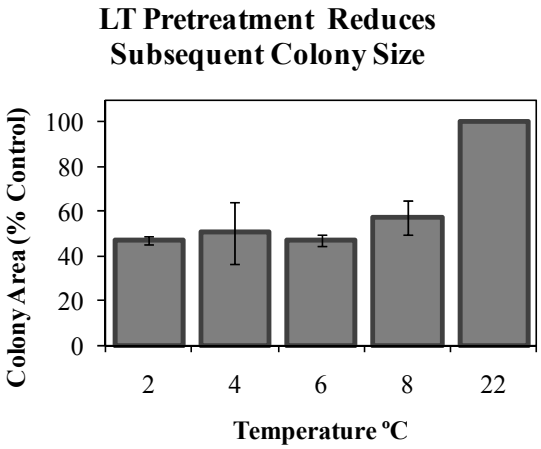

Figure 1: Low temperature pretreatment of leaves prior to powdery mildew infection reduces overall colony development. This effect is not dependent on the degree or duration of an exposure below 8C, just the simple occurrence of an LT event.

Pre-inoculation LT treatment also impaired the initial infection and establishment of *E. necator.* While there was no significant differences between the duration (2 or 8 h) of a given temperature treatment, there was a significant reduction in infection efficiency at colder temperatures. The 2°C treatment had significantly more conidia in category (i) compared to the 8C treatment, but the 8°C treatment had significantly more conidia in category (iii). The 4 and 6°C treatments were not significantly different from each other or the 2 and 8°C treatments for all

categories (Tukey's HSD, significance at P<0.05). All low temperature treatments had significantly less conidia in category (iii) than the control. Conidia failing to develop branched hyphae after 48 h on the host tissue are considered impaired in development. Having a high proportion of conidia in a treatment falling into category (i) indicate a failure to infect and therefore, a failure to establish an infectious colony. Ultimately, this suggests that LT treatments are inducing a host resistance that is likely acting at the infection stage of an epidemic.

Residual effects of pre-inoculation LT treatments on establishment of conidia and suppression of colony development. To test whether LT-induced resistance was permanent or transient, similar experiments as above were repeated, using highly susceptible leaves from 5-year-old potted *V. vinifera* 'Cabernet franc'. However, there was only a single treatment of 8 h at 2°C. Leaves were the inoculated with either a conidial suspension or via dusting as described above, at 12, 24, 36, or 48 h post cold treatment. Observations of germinated conidia or nascent colonies were made at 48 h or 5 dpi as described above. This allowed us to establish a timeline of responses during cold-induced disease resistance.

Cold-induced disease resistance was transient in normally susceptible grapevine tissue, peaking between 24 and 36 h after LT treatment, depending on the response observed (Figure 2). The 24 h and 36 h treatments resulted in mean colony sizes that were significantly less than the control (Dunnett's Method, P<0.0001 and P=0.0014, respectively), while the 12 and 48 h treatments were not significantly smaller than the control. Significantly more conidia in the 24 h treatment failed to infect (26%) compared to the control (11%, P= 0.007). The other treatments (12, 36 and 48 h) were not significantly different from the control, with 11, 11 and 13% of conidia failing to infect, respectively. The 24 h treatment had significantly less conidia developing branched hyphae compared to the control (52 vs. 81%, P=0.015), while the other treatments at 12, 36 and 48 h were not different than the control. These results indicate that cold-induced disease resistance is neither immediate nor permanent, but transient.

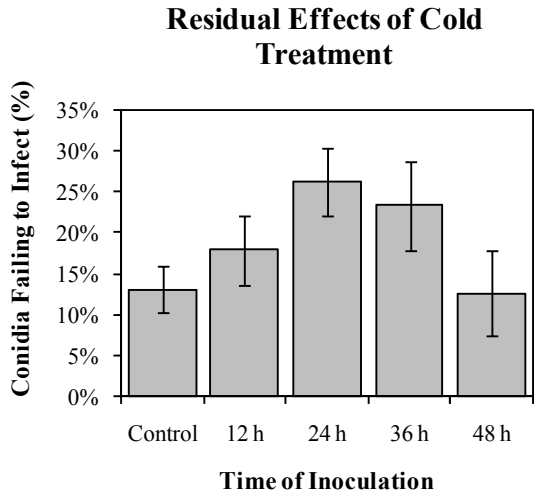

Figure 2: Cold induced resistance is transient, with a maximum resistance occurring between 24 and 36 h after low temperature exposure. Effects on initial infection are shown here, with an increased number of conidia failing to infect leaf tissue after 48 h.

Effects of post-infection exposure to low temperature on development of nascent mildew colonies. We also wanted to determine if LT could impair extant powdery mildew colonies. Ontogenically-susceptible leaves were detached from 5-year-old potted *V. vinifera* 'Pinot noir' were dusted with conidia, and nascent colonies aged 2, 3, 4, 5, or 6 days were then exposed to an LT treatment of 2°C for 8 h. Twenty-four hours post-LT treatment, nascent colonies were stained with fluorescein diacetate, and after 3 min colonies were viewed under fluorescence microscopy (235 to 500 nm excitation filter), and percent of colony area not exhibiting bright green fluorescence was visually estimated. Exposure of nascent colonies to LT increases hyphal mortality (Figure 3), but the significance of the effect is related to colony age at the time of exposure. Both the 3- and 4-day-old colonies suffered the most significant damage (66.7 and 57.2% more hyphal mortality than controls, with Student's t-test at P=0.005 and 0.07, respectively), while the 2-day-old colonies suffered the least damage (11.6% more hyphal mortality than the control, P=0.71), and the 5- and 6-day-old colonies were in between, at 49.9 ad 32.1% more hyphal mortality than the controls (P=0.13 and 0.16, respectively). This is interesting, as 3- and 4-day-old colonies, when grown in ideal conditions, are generally at the stage of transitioning from vegetative to reproductive growth. Previous studies have indicated that the sporulation signal for *E. necator* is propagated from the center of a colony, and if hyphal tips are removed prior to having the signal reach them, then their sporulation 'clock' is reset (Gadoury *et al.*, 2004). This may explain the extended latent periods we observed in early-season colonies developing in the field; the LT events may be causing enough hyphal damage to reset the sporulation clock within a colony.

Differences between nighttime ambient and leaf surface temperature. Radiational cooling of the leaf surface may lead to a drastically different microclimate experienced by developing powdery mildew colonies than what would be suggested from ambient air temperature measurements. To test this, we measured leaf surface temperature in an established 'Chardonnay' plot at the New York State Agricultural Experiment Station in Geneva, New York, USA, but taking direct leaf surface temperature measurements using an IR gun, and measuring ambient air temperature 2 cm above the leaf surface using thermocouples. Measurements were taken 30 min after sunset and 30 min before sunrise on the evenings of 12, 17, and 18 May 2009.

On cloudless, windless nights, leaf surface temperatures at 30 min after sunset can super cool 3.8 to 7.6°C cooler than ambient air temperatures (both significantly colder at P=0.01, Student's t-test). Just prior to sunrise, leaf temperatures were still 3.0 to 3.4°C colder than ambient air (significantly colder at P=0.01, Student's t-test). This difference in temperature was reduced during breezy or overcast nights, when leaves were only 0.9°C colder. On the morning of 19 May 2009, just prior to sunrise, leaf surface temperatures actual reached -1.7°C (se=0.5) and frost damage occurred, while the ambient air temperature was reading 1.7°C (se=0.1).

General Conclusions. We demonstrated that there is a previously unaccounted for *negative* effect of low

temperatures on powdery mildew viability, and a potential positive impact of short-term cold-induced disease resistance in *V. vinifera*. Naturally occurring cold events could, and probably do produce the observed responses, not only in what are stereotypically thought to be cold viticultural regions, but in some of the warmest grape-growing regions of the world (Table 1). Accounting for the observed effects of acute low temperature exposure upon both the host and pathogen may help improve forecasting of powdery mildew, particularly early in the growing season.

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Table 1: Summary of overnight low temperature events between budbreak and bloom occurring in five viticulture regions.

Site	Climate Type	Ave. min. temp. (°C)[x]	Days between BB and BLwith min. temp. ≤ 6°C[y]
Geneva, New York	Cool temperate	7.1	**17**
Hobart, Tasmania	Maritime	6.0	**21**
Davis, California	Mediterranean	7.6	**16**
Bernkastle, Germany	Northern temperate	7.5	**18**
Loxton, South Australia	Mediterranean/ desert	8.9	**17**

[x] Average daily minimum temperature of the period between budbreak and bloom.
[y] Integer average of period from budbreak to bloom for 2005 to 2007 inclusive.

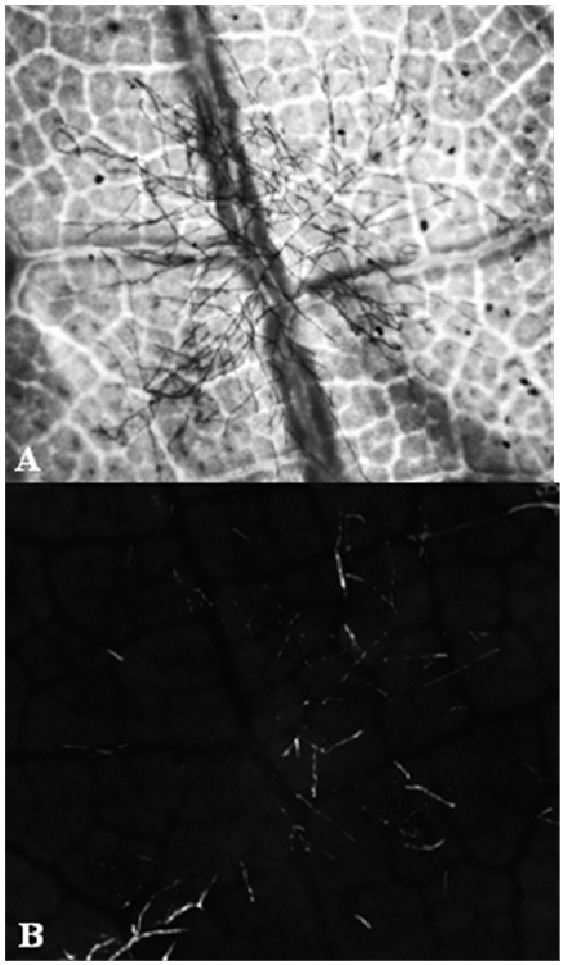

Figure 3: Low temperature exposure to nascent colonies can cause significant damage. A) A 3-day-old colony exposed to cold looks healthy under a microscope, but B) when viewed using FDA vital stain, a large portion of the colony is dead

Effect of Prior Vegetative Growth, Inoculum Density and Light on Conidiation in *Erysiphe necator*

D. Gadoury[a], LM. Wakefield[a], RC. Seem[a], L. Cadle-Davidson[b], IB. Dry[c]

[a]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA. [b]USDA-ARS Grape Genetics Research Unit, Geneva, NY 14456, USA. [c]CSIRO Plant Industry, Horticulture Unit, University of Adelaide, Waite Campus, Adelaide, SA 5064, Australia.

Introduction

A driving force in epidemics of grape powdery mildew is the abundant production of conidia. Thus, disruptions in the timing or volume of conidiation could conceivably slow or even stop the progress of disease. Increased understanding of the process of conidiation might allow for direct manipulation of the process in the field. Additionally, knowledge of conditions that affect the latent period or trigger the production of conidia would be useful in the construction of improved forecasting models, thus allowing for more precise timing of fungicides. Previous investigations into the onset of asexual sporulation in *E. necator* have generally focused on rate - determining relationships between temperature, relative humidity, and latent period, as well as quantitative effects of the above factors upon numbers of conidia produced and their survival (Delp, 1954; Pearson and Gadoury, 1992; Carroll and Wilcox, 2003). However, the factors which signal and control the initiation of conidiation itself remain poorly understood. Our objective was to better define the factors involved in the qualitative change that occurs when a mildew colony switches from vegetative growth to sporulation.

Materials and Methods

Microsurgery experiments. Experiments were conducted at the Commonwealth Scientific and Industrial Research Organisation in Adelaide, Australia (CSIRO), and at the New York State Agricultural Experiment Station in Geneva, NY USA (NYSAES). Clonal isolates at each location were prepared as previously described (Gadoury and Pearson 1991). For each repetition of the experiment, 8 or 10 *Vitis vinifera* seedling or 'Chardonnay' leaves were inoculated by dispensing 5 µl droplets of suspensions containing 10^5 conidia/ml of a clonal isolate onto each side of the midvein using a digital pipette. At 3 and 4 days after inoculation, the edges of four colonies were marked at four points with a finepoint indelible marking pen. Twentyfour hours later, i.e., 4 or 5 days after inoculation, the center of the colony as defined by the area within marked points was excised. Uncut colonies were reserved as controls. Each day following excisions, the control colonies and the remaining colony margins were observed for signs of sporulation.

Effect of Inoculum Density on Latent Period. Conidia from clonal isolates of *E. necator* were rinsed from freshly sporulating colonies using distilled water plus 0.05% Tween 20 to prepare a spore suspension containing 10^5 conidia/ml. The forgoing was immediately diluted to yield a series of eight conidial suspensions containing 4, 8, 16, 32, 64, 128, 256 or 512 conidia/5 µl drop. Detached seedling or Chardonnay leaves received one 5 µl drop of each concentration of spore suspension, droplets. Germination potential of the suspension in each repetition of the experiment was recorded. The density of inoculation was determined from the area of the spore deposit resulting from the 5 µl droplets (mean of 4.43 mm²) adjusted for the percentage of germinating conidia within each prepared conidial suspension. Starting four days after inoculation colonies were observed twice daily for signs of sporulation.

Effect of Light on Sporulation. Grapevine seedlings were grown from seed harvested from *V. vinifera* 'Riesling' as previously described (Gadoury and Pearson 1991).

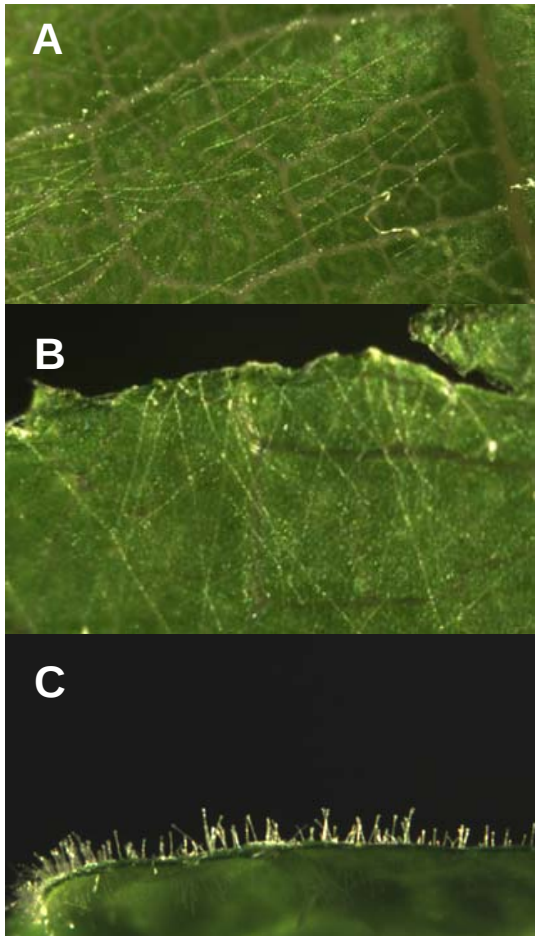

Figure 1: (A) Pre-sporulation hyphal growth at margin of 4-day old mildew colony (B) Continued radial growth of mildew colony without sporulation on day 7 following excision of colony center on day 4 (C) Sporulation at margin of mildew colony on day 7 following excision of center of mildew colony on day 5. Colonies that were not surgically altered also sporulated on day 7.

Detached leaves from seedlings were surface sterilized and placed on 1% agar plates. Each leaf was inoculated with five 5 µl drops of a conidial suspension containing approximately 250 conidia/drop. Colonies were allowed to develop under 12 hour day/night cycles at 22°C. Thirty-six hours after inoculation, leaves were divided into three groups: one receiving 24 hours of light per day, one receiving 12 hours of light, and one receiving no light. Starting on day 5, sample leaves were removed and observed at 32 X magnification for signs of sporulation. On day 8, leaves from all three groups were examined at 32 X magnification and the number of conidiophores/cm^2 at the center of the colony was recorded. Conidial production was also assessed by lightly touching glass microscope slides to colonies and then counting the number of conidia adhering to the slides at 100 X magnification. Finally, on day 8, colonies borne on leaves which had received no light were exposed to a 12 h day/night cycle at 22°C, and conidiophore and conidial production was assessed as above 24 h later on day 9. Within each light treatment, a total of 15 to 20 leaves were sampled and the experiment was repeated a total of four times.

Results

Microsurgery Experiments. Excision of the colony center at 4 days post inoculation (DPI) delayed sporulation in the remaining hyphae until 9 to 10 DPI (Figure 1). Colonies whose centers were excised on day 5 after inoculation sporulated on day 7 following inoculation, similar to non-excised control colonies (Figure 1).

Effect of Inoculum Density on Latent Period. The length of the latent period of the clonal isolates at CSIRO and NYSAES varied from a maximum of approximately 9 days to a minimum of 5 days depending upon the density of inoculation (Figure 2). The latent period decreased exponentially as the number of germinable conidia increased above 1 per mm² until inoculation density reached approximately 10 to 20 germinable conidia per mm² (Figure 2). Increasing the conidial density from 25 conidia/mm² to 250 conidia/mm² produced only a slight decline in the duration of the latent period (Figure 2).

Effect of Light on Sporulation. Light was necessary for initiation of sporulation. Approximately 10% of colonies exposed to 12 h day/night cycles or continuous light sporulated on day 5 and 100% had sporulated by day 7. Colonies allowed to grow under a normal day/night cycle for 36 hours but thereafter kept in darkness did not sporulate on day 5 or on day 7. However, when colonies that had been incubated in darkness until day 7 were thereafter exposed to a 12 h day/night cycle, conidiophores developed at the margin of the colony on day 8, and mature conidia were produced on day 9. Thus, colonies incubated in darkness were competent for sporulation, but required light to initiate the process.

Discussion

The requirement of a period of asexual growth before reaching competence for sporulation has been previously demonstrated in *E. necator* and other fungi, (Adams et al 1998; Bailey-Shrode and Ebbole 2004, Pearson and Gadoury 1992). In the case of *E. necator,* approximately 6 to 9 days after inoculation, conidiophores appear singly and in small groups throughout colonies, including the margin where the hyphae are less than 48 h old. The foregoing is consistent with production of a signal that coordinates appearance of these structures throughout the body of the colony.

The microsurgery experiments provided further evidence that initiation of asexual sporulation is triggered through a coordinating signal. Five-day-old colonies wherein all hyphae except those at the colony margin were excised began to sporulate 7 days after inoculation, in time with unaltered controls. However, sporulation was greatly delayed in four-day-old colonies subjected to the same treatment. In effect it seems the sporulation clock is reset by removing the colony center on day 4, but by day 5, the sporulation process has been initiated and cannot be reversed by excising the colony centers.

Increasing inoculum density was shown to decease latent period, suggesting that colony density may be the operative factor driving promulgation of the sporulation signal, and that the critical density might be achieved either through time or by an increased initial density. A similar process has been suggested for *Aspergillus nidulans*, wherein the *flu*G gene produces a small diffusible factor consistently associated with sporulation (Lee and Adams 1996). Although quorum sensing phenomena have not been well characterized in fungi, other morphogenesis events in fungi have been similarly linked to density responses (Hornby et al 2001, 2004; Ramage et al 2002; Nickerson et al 2006). If signaling plays an active role in asexual sporulation in *E. necator*, disrupting the pathways involved would provide a novel means of control.

Our findings also have implications for the design of field and laboratory experiments related to forecasting development of powdery mildew epidemics. Previous experimental inoculations have generally involved the transfer or application of large numbers of conidia to host tissues to ensure successful infection and establishment of the pathogen. This is in contrast to infection resulting from airborne conidia in a natural setting where colonies might be dependent upon the success or failure of a single conidium to become established. Thus, latent periods and estimated rates of epidemic development in extant models may require adjustment if based upon experimental inoculations using unrealistically high numbers of conidia.

Density dependent development of *E. necator* might also explain why diffuse and non-sporulating infections are commonly found on berries infected during the transitional stages between moderate ontogenic susceptibility and near-immunity (Gadoury et al 2007). This colony phenotype would be consistent with the failure to reach a critical colony density required to promulgate a signal for sporulation, thus keeping the colonies in their observed, diffuse, and purely vegetative state.

Light appears to function as a trigger for asexual sporulation in *E. necator* once colonies reach a critical density. The role of light as a modifier of asexual development is a common theme in filamentous fungi (Purschwitz et al, 2008; (Sánchez-Murillo et al, 2004; Kumagai, 1989; Casas-Flores et al, 2006). More recently, Suthparan et al (2010) reported that conidiation in the rose powdery mildew pathogen *Podosphaera pannosa* was stimulated by blue light (420 to 520 nm) but repressed by red light (620 to 720 nm). The rapidity with which sporulation can be induced upon exposure of dark-suppressed, but sporulation-competent colonies of *E. necator* to light may provide a model system for the study of metabolic pathways operational in the process wherein gene expression and regulation can be examined in a temporally precise fashion in sporulation-competent

colonies during the minutes before and after light exposure.

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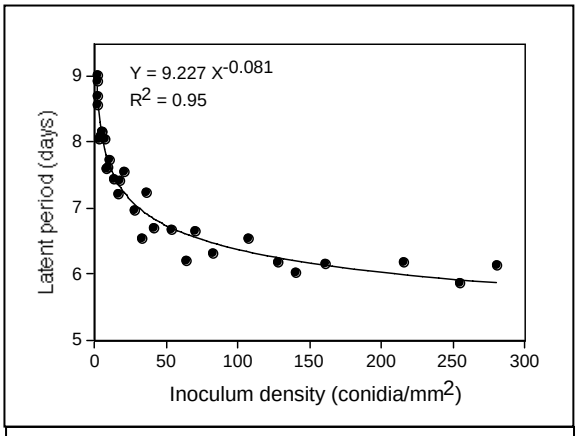

Figure 2: Effect of inoculation density on latent period of *Erysiphe necator*. Leaves were inoculated with clonal isolates collected in Geneva, NY, USA; and were observed daily for signs of conidiation. Similar results were obtained using a clonal isolate from Adelaide, Australia.

Dynamics of ontogenic resistance and growth variation in the interaction powdery mildew-grapevine

S. Schnee, J. Jolivet, A. Calonnec

INRA-Bordeaux, UMR INRA-ENITA 1065 Santé Végétale, BP 81, 33883 Villenave d'Ornon, France

Introduction

Powdery mildew is an ubiquitous fungal disease of cultivated grapevine (*Vitis vinifera* L.). The causal agent, *Erysiphe necator* Schwein., is an obligate biotrophic pathogen producing specialised infection structures in host epidermal cells to take up nutrients. Favourable environmental conditions insure repeated asexual reproduction, involving foliage and berries damage. In the vineyard, epidemic development is initiated by primary infections on young vegetative shoots, resulting from the dispersion of ascospores after cleistothecium dehiscence and/or conidia from latent mycelium conserved in dormant buds (Rumbolz *et al*., 2000). The extent of the following epidemic is closely linked to the dynamic interaction between the pathogen, the host and their environment (Deytieux-Belleau *et al*., 2009). Environmental factors influence the biological process, generating simultaneous fungal and vegetative growth. On one hand, the plant host produces a vegetative biomass, defined by a growth rate and a maximum potential quantity of tissues. On other hand, pathogen development and dispersion depend on infection efficiency linked to the pathogen's fitness, the ability to produce an important quantity of dispersal spores, and the probability of contact with a neighbouring organ in space and time. However biotrophic pathogens are strongly dependant on subtrate quality, defined by a quantity of susceptible tissues. Ontogenic resistance defined by the intrinsic acquisition of resistance with increased organ age, is a key factor limiting repetition of the infection process. Therefore the window of pathogen colonization is restricted to the range of susceptible leaves, determined by vegetative growth of the host plant. Consideration of the dynamic change of crop architecture in plant disease management constitutes an alternative integrated strategy to counteract epidemic development (Calonnec *et al*., 2009; Schnee *et al*., 2010). To date, main investigations of ontogenic changes have been undertaken on the grape berry (Ficke *et al*., 2002), but relatively little information is available on the mechanisms associated with leaf resistance-age dependence (Doster and Schnathorst, 1985). On the model powdery mildew-grapevine, we investigated 1) the dynamics of the ontogenic resistance mechanism on leaves, 2) the effect of host growth variation on the susceptibility of foliar tissues to powdery mildew infection.

Methods

Two sets of data were analysed. 1) *For the investigation of ontogenic resistance mechanism*: This experiment was carried in 2005 (experimental site of Couhins) on shoots sampled at two different dates during the vegetative growth of grapevines cv. Cabernet Sauvignon (May 12th, stage 5-6 leaves; May 26th, stage 10-12 leaves). 2) *For the study of the impact of a plant growth variation on ontogenic resistance:* This experiment was carried in 2009 (experimental site of Latresne) on shoots sampled from vine-stocks localized in different vigour areas (cv. Merlot). For the two experiments, the following methodology was performed.

Shoots were cut early in the morning and immediately brought back to the laboratory. Before sampling, leaf petioles were marked with a colour code to specify their respective position on the shoot. In axenic conditions, leaves were disinfected (in a sterile water bath containing 65% calcium hypochlorite) and rinsed in sterile water. Three foliar discs (Ø 22 mm) were cut in each leaf to be distributed in 3 different Petri dishes corresponding to three pathogenicity tests. Six foliar discs (Ø 8 mm) were cut at the same time for glucose analysis. Leaves from the same foliar level were distributed in Petri dishes (6 discs from 6 shoots per dish), containing an agar medium (20g l^{-1}) supplemented with benzimidazole (30 mg l^{-1}). Petri dishes were placed in a settling tower and were artificially inoculated by blowing conidia from a 14 day infected leaf, according to Cartolaro (1990). All leaf levels were inoculated together in one settling tower. The infection capacity was assessed 72 hours post inoculation (hpi) by counting conidial development stages, after removal of fungal structures by a scotch application and cotton blue staining procedure. After inoculation of each foliar disc by deposition of a few conidia using a needle, the colony growth was measured 4, 7, 10 and 13 days post inoculation (dpi). Sporulation assessment was performed by measuring the quantity of generated conidia 12 dpi (first date of sampling) and 14 dpi (second date of sampling) in the 2005 experiment and 13 dpi in the 2009 experiment. Sporulation was quantified by using a particle counter (Beckman Coulter) that recorded cells sized 18 to 35 µm. Determination of glucose and starch concentration in the foliar discs was performed by enzymatic microdosage (kit Biosentec). Data were statistically analysed with Prism software.

Results

Dynamics of ontogenic resistance in relation to foliar age

The appearance of ontogenic resistance was assessed by comparing the intensity of sporulation per infected leaf surface in relation to the leaf age.

In 2005, the two dates of sampling presented an identical optimum of sporulation for leaves 5-6 days old. Older leaves displayed decreased sporulation in both cases (Figure 1). The amplitude of inoculum produced was two fold higher at the late sampling date. The range of cell glucose content varied from less than 1 (% in dry weight) for the young leaves to more than 4 for the older leaves. Simultaneously with the sporulation decrease, leaves 8-9 days old displayed a drastic increase in cell glucose content. This trend in the susceptibility tissue pattern was also observed for infection efficiency (data not shown).

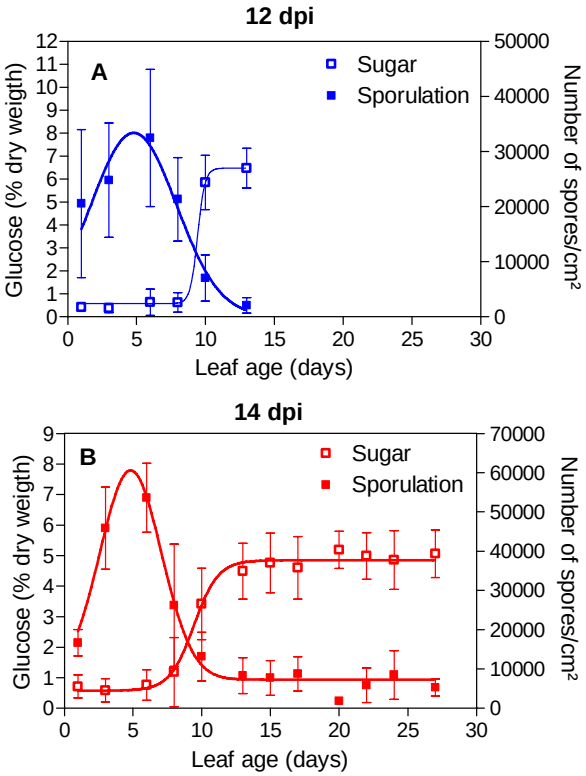

Figure 1: Intensity of sporulation and glucose cell content in relation to foliar age. Shoots were sampled at phenological stages "5-6 leaves" (May 12th) (A) and "10-12 leaves" (May 26th) (B). Each point corresponds to an average of 6 repetitions, and bars indicate standard errors.

Effect of host growth on the susceptibility of foliar tissues

The effect of vine development on tissue susceptibility was measured by comparing the intensity of sporulation per infected leaf surface in relation to leaf age. The maximum amplitude of sporulation was similar between the two levels of vigour (Figure 2). However, the range of age of sporulating leaves was significantly lower at the low vigour level (from 4 to 10 days old) than at the high vigour level (from 4 to 15 days old). The age for maximum sporulation was also significantly earlier at the low vigour level (between 6-8 days old versus 8 to 12 days old at the high vigour level). The glucose concentration increased with the leaf age and a clear distinction appeared for the 12 day old leaves between the two levels of vigour (Figure 3). The observed variability at some points could be attributed to individual dynamics of the selected shoots.

Discussion

This study showed that the establishment of ontogenic resistance with regard to foliar age is correlated with a steep increase in cell glucose concentration. Moreover ontogenic resistance appearance is linked to host vegetative growth performance.

On grapevine, foliar tissue susceptibility occurs on the youngest leaves and is rapidly modified by the appearance of ontogenic resistance. Mechanisms of ontogenic resistance establishment remain hypothetical and the investigation of associated physiological factors could allow a better understanding of the appearance of this age-related resistance. Young leaves are considered to be a sink organ corresponding to a limited activation of the photosynthetic process. The time of physiological maturity of the leaf appears to be approximately between 6 and 8 days old, when cellular glucose content increases. This physiological leaf evolution corresponded to the progressive decrease in sporulation. The underlying causes of the observed relationship between a restricted range of sporulation and an increase of leaf glucose content with foliar age are subject to speculation, but sugar accumulation is known to modify osmotic value that can promote resistance to fungal penetration (Schnathorst, 1959).

A low vigour may modify the susceptibility of tissues, by an earlier appearance of ontogenic resistance at a fixed foliar age. This trend was already observed in different experiments, in which vigorous vines had a significantly higher number of diseased leaves (Schnee *et al*., 2010) and a higher percentage of diseased berries (Valdes, 2007). The plastic behaviour of grapevines supports the possibilities of alternative host growth management to limit epidemic progress.

Further experiments in 2010 will be performed to: 1) explore determinism of the ontogenic resistance in relation to dosage of secondary metabolism compounds; 2) understand the ontogenic resistance dynamics with regard to different host growth; and 3) test a model of causal relationship between variables by PLS-path modelling (Tennenhaus *et al*., 1999) to identify variables that contribute significantly to an explanation of the pathosystem interaction.

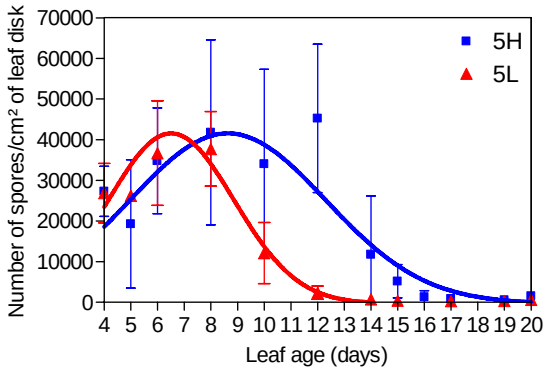

Figure 2: Sporulation level in relation to leaf age and associated vigour level (5H: high vigour level, 5L: low vigour level). Each point corresponds to an average of 6 repetitions, and bars indicate standard errors.

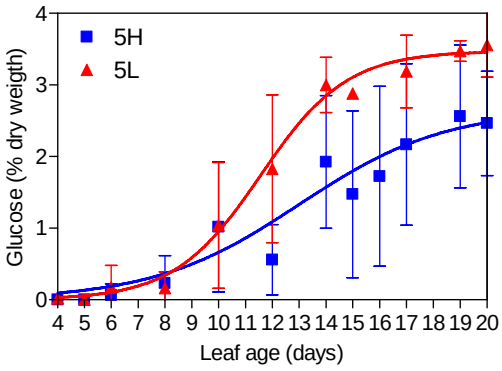

Figure 3: Glucose cell content in relation to leaf age and associated vigour level (5H: high vigour level, 5L: low vigour level). Each point corresponds to an average of 6 repetitions, and bars indicate standard errors.

Acknowledgements
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Maximum severity of powdery mildew on grape leaves coincides with the sink to source transition

A.M. Smith, K.J. Evans, R.Corkrey, S.J. Wilson

Tasmanian Institute of Agricultural Research, University of Tasmania, New Town Research Laboratories, 13 St Johns Avenue, New Town, Tasmania 7008, Australia.

Introduction

Variation in the extent of pathogen colonisation according to leaf position has been reported for a wide range of biotrophic pathogens infecting woody, perennial plants. Doster and Schnathorst (1985) reported that the percentage of germinated *Erysiphe necator* conidia that developed hyphae, 48 h after inoculation of grapevine leaves, declined as leaves matured beyond a mid-vein length of 5 cm. There was also a corresponding decline in colony hyphal length. The mechanism of ontogenic resistance in leaves, including those of grapevines, remains unknown. A first step in understanding ontogenic resistance is the definition of leaf developmental characters associated with maximum colonisation by *E. necator*. The carbohydrate status of leaves was selected for this study because powdery mildew fungi derive their nutrition from living host cells and act as sinks for plant photosynthates. As leaves develop, they convert from being a net importer (sink) to a net exporter (source) of carbohydrate (Turgeon & Webb, 1973). The timing of the transition from leaf photosynthetic sink to source is correlated with attainment of a positive carbon balance: import stops and export begins when the supply of carbohydrates exceeds the growth and respiratory needs of the leaf (Pate & Atkins, 1983).

The objective of this study was to test the hypothesis that the leaf on a primary grapevine shoot that expresses maximum severity of powdery mildew is the one that was infected just when the leaf ceased importing carbohydrate. This paper describes identification of these leaves on primary shoots of Cabernet Sauvignon vines. A Bayesian model (Gelman *et al.*, 1996) was constructed to quantify the non-linear change in powdery mildew severity as a function of increasing leaf position on primary shoots with different rates of leaf emergence.

Methods

Dormant cuttings of *Vitis vinifera* L. cv. Cabernet Sauvignon, with roots, were grown in 15 cm-diameter pots (one cutting/pot), pruned to one bud. Ten plants were grown at 25°C (± 5°C) and then the experiment was repeated in the same glasshouse except that eight plants were grown at 18°C (± 8°C). These two pre-inoculation environments were intended to result in relatively fast (near optimum) or slow (sub-optimum) rates of leaf emergence, estimated from linear regression of the plastochron index (30 mm reference length of the lamina) against calendar day (Schultz, 1992). When shoots had developed approximately 20 leaves, a suspension of 10^5 *E. necator* conidia per ml water was applied to the adaxial side of all leaves using a hand held atomizer and plants were incubated in the glasshouse at 25°C (± 5°C). After 14 days, disease severity per leaf was estimated visually as the percentage of leaf area colonised by *E. necator*.

Another ten plants were grown in the glasshouse at 25°C (± 5°C) and all leaves inoculated by transfer of dry conidia with an artists' paint brush. At 72 h after inoculation, leaf sections were fixed and stained before using light microscopy to determine the germination status of 40 conidia per leaf section. The presence of one of more secondary hyphae per germinated conidium was used to indicate that infection had proceeded to penetration of the leaf cuticle (Ficke *et al.* 2003). For each shoot, the modal leaf position at maximum disease severity or maximum percentage of conidia with secondary hyphae was identified by a bootstrap approach (Efron & Tibshirani, 1993).

Bayesian analysis

Pathogen growth model: The proportion of leaf area covered by powdery mildew was estimated by the logistic model: $a_{jp} = 1/(1+ \exp(\beta_j - \gamma_j p))$ (1), where a_{jp} was the proportional area colonised by mildew on leaf position (p) on plant (j), and α_j and β_j were constants to be estimated. The leaf position was the ordinal series $p = 1,2,\ldots p_j$ in which $p = 1$ had a lamina length ≥ 30 mm and the length of the series could differ between plants. The condition $\alpha_j > 0$ was imposed to ensure that a_{jp} increased with leaf position. The constant γ_j was an indicator of the rate of colonisation and γ_j indicated the magnitude of colonisation.

Leaf resistance model: Leaf resistance to powdery mildew was estimated as: $s_{jp} = 1 - (1 - \exp(-p\delta_j))^{\varepsilon_j}$ (2), where s_{jp} was the disease resistance for leaf position p on plant j, and δ_j and ε_j were constants to be estimated. In this model, the magnitude of s_{jp} declined as the leaf position p (as defined for equation (1)) increased, meaning that resistance increased as leaves aged. The conditions $\delta_j > 0$ and $\varepsilon_j > 0$ were imposed to ensure that s_{jp} decreased with leaf position. The constant δ_j indicated the rapidity of the disease resistance response with increasing leaf position and ε_j indicated the position at which leaves expressed an equivalent level of disease resistance.

The overall model of disease severity as a function of leaf position was described using the Normal distribution in which the mean was given by the product of the two models: $m_{jp} \sim N(a_{jp} \times s_{jp}, \tau_m)$ (3), where τ_m was the reciprocal variance or precision. Probability distributions ('priors') were assigned to the parameters β_j, γ_j, ε_j, δ_j, with separate means for the two temperature groups, and a single prior for τ_m. The means of the priors for β_j, γ_j, ε_j, δ_j are referred to as overall means. The remaining steps in the Bayesian analysis and model validation will be presented.

Identification of the sink to source transition

A further ten and eight additional plants were grown at average temperatures of 25°C and 18°C, respectively. When these plants had developed approximately 20 leaves, two 'source' leaves for carbohydrate were enclosed in a polythene bag and treated with $^{14}CO_2$ at 08:00. Photosynthesis was allowed to continue for 2 h before removal of the polythene bag. After 24 h, leaves exposed to $^{14}CO_2$ and all leaves distal to the exposed leaves were cut from the shoot and dried for 7 days in a plant press at room temperature. Dried leaves of each shoot were then exposed to general-purpose X-ray film in a standard medical X-ray cassette for 3 weeks prior to film development. After inspection of the autoradiographs, the leaf position for the cessation of the sink to source transition was designated as the first (proximal) leaf showing no visual evidence of ^{14}C accumulation.

Results

On average, 0.54 leaves emerged per day for shoots exposed to the 25°C pre-inoculation environment and 0.23 leaves emerged per day for shoots exposed to 18°C. For both pre-inoculation environments, powdery mildew was more severe on leaf positions 3–5 than on younger (positions 0–2) or older leaves (positions 6 or higher, Figure 1). No powdery mildew was observed at leaf positions ≥ 17 on shoots with the higher rate of leaf emergence, nor at leaf positions ≥ 11 for shoots with the lower rate of leaf emergence (Figure 1). On average, the higher rate of leaf emergence resulted in a greater proportion of diseased leaves per shoot and a higher disease severity per leaf position (Figure 1).

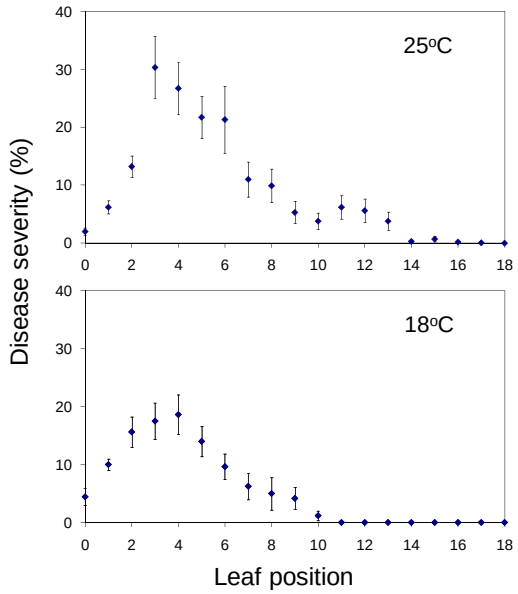

Figure 1: The effect of leaf position (values increase as leaves age) on shoots of glasshouse-grown Cabernet Sauvignon vines on the mean severity of powdery mildew 14 days after the determination of leaf position and inoculation. Plants were grown at an average temperature of 25°C or 18°C prior to inoculation. Error bars represent the standard error of the mean.

The Bayesian model obtained a good fit for all shoots (for example, Figure 2). The magnitude of initial colonisation and the rate of colonisation, indicated by β_j and γ_j respectively in the pathogen growth model, were both greater on shoots with a faster rate of leaf emergence before inoculation. The probability that the overall posterior mean of β_j and γ_j for vines with a lower rate of leaf emergence was less than the overall posterior mean for the 25°C pre-inoculation was 1.0 and 0.98, respectively. For the leaf resistance model parameters δ_j and ε_j, the probabilities that the overall posterior mean at the slower rates of leaf emergence were less than the overall posterior mean at the faster rate were 0.117 and 0.287, respectively.

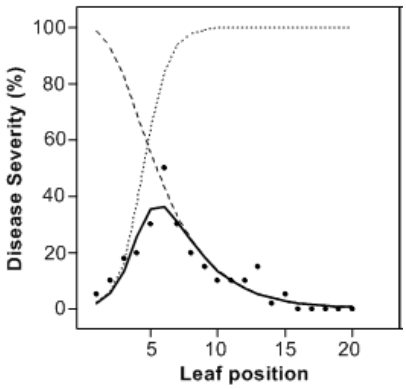

Figure 2: Example of the 'goodness of fit' of the Bayesian model for data from one grapevine shoot grown at an average temperature of 25°C prior to inoculation with *E. necator*. The curves fitted to observed data (circles) are the overall model (–), the leaf resistance model (- - -) and the pathogen growth model (…..).

The modal leaf position at maximum disease severity occurred on average at leaf positions 3.7 and 4.7 for the 18°C and 25°C pre-inoculation environments, respectively, with the latter corresponding to the mean modal leaf position of 4.5 for the maximum percentage of conidia germinating to form secondary hyphae. The shape of the response for the percentage of conidia germinating to form secondary hyphae as a function of leaf position was similar to that for disease severity. The penetration of epidermal cells by germinating *E. necator* conidia declined as leaves matured beyond leaf position 4 (data to be presented).

There was a clear association between the leaf position for maximum severity of powdery mildew and the position of the leaf completing the sink to source transition for shoots exposed to either pre-inoculation environment. The modal leaf position for the leaf completing the sink to source transition occurred on average at leaf positions 3.8 and 4.7 for the 18°C and 25°C pre-inoculation environments, respectively.

Discussion

The expression of powdery mildew on grape leaves was clearly different in plants exposed to different pre-inoculation environments. Unlike previous studies, Bayesian analysis described how disease severity initially increased and then decreased due to leaf ontogenic resistance. The analysis indicated that there was no significant difference between plants grown in the two different environments for the parameters of the leaf resistance model, yet there was a significant difference for the pathogen growth model. Therefore, mechanistic modelling enabled these effects to be separated. Either the pre-inoculation environment had a direct effect on the

nutritional quality of the plant tissue to be colonised by *E. necator* and/or there was a direct effect of the environment, perhaps temperature, on the expression of pre-formed and/or induced mechanisms of resistance. Interpretation of the Bayesian analysis tended to support the former hypothesis more than the latter.

Leaves of Cabernet Sauvignon vines were most prone to development of severe powdery mildew immediately after leaves had ceased to import carbohydrate. This relationship was maintained when plants were subjected to different pre-treatment environments. Low disease severity on newly unfolded leaves that were 'sinks' for carbohydrate may have been the result of a number of factors including rapid expansion of leaf area, a lack of secondary metabolism for defence and possibly a pathogen that is less able to compete with the sink strength of the host. An ideal ecological niche for infection by *E. necator* might be created immediately after completion of the sink to source transition due to high amounts of the right form of available sugar (Sutton *et al.*, 1999). Future studies investigating mechanisms of ontogenic resistance in relation to the photosynthetic sink to source transition will require greater spatial and temporal resolution of this process in leaves.

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Photosynthetic activity in grape leaf tissue with latent, visible and 'virtual' downy mildew lesions

T. Caffi[a], S.E. Legler[a], V. Rossi[a], S. Poni[b]

[a] Università Cattolica del Sacro Cuore, Istituto di Entomologia e Patologia Vegetale, I-29122 Piacenza, Italy.
[b] Università Cattolica del Sacro Cuore, Istituto di Fruttiviticoltura, I-29122 Piacenza, Italy

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Plasmopara viticola Berl. & De Toni is the causal agent of grapevine downy mildew, a major disease in grape-growing areas with temperate climates and rainy springs. *P. viticola* epidemics are characterized by sexual and asexual infection cycles that partially overlap for a part of the season (Rossi *et al.*, 2008). Infection processes and disease symptoms caused by sexual and asexual spores are similar, and determining whether an infection was caused by sexual or asexual propagules requires molecular analysis (Gobbin *et al.*, 2005). Zoospores swim in the water film on the abaxial side of leaves and when they reach stomata, they germinate and produce a germ tube that penetrates into the host tissue. The mycelium colonizes the leaf mesophyll and forms haustoria that biotrophically parasitize the host cells. At the end of an asymptomatic incubation period, the first disease symptoms appear on the upper surface of the leaf as pale-yellow lesions (called 'oil spots'); the pathogen then produces sporangiophores that grow through stomata on the underside of the leaves (Agrios, 1988; Lindenthal *et al.*, 2005).

Downy mildew causes direct losses, by rotting inflorescences and clusters, and indirect losses, by affecting photosynthetic activity and transpiration of the infected leaf tissue and by inciting premature defoliation of vines. All of these reduce yield (Jermini *et al.*, 1998).

There is increasing interest in the modelling of plant–pathogen relationships to estimate potential yield losses caused by the disease (Madden and Nutter, 1995). These models usually simulate the development of the leaf area, its photosynthetic activity, and the partitioning of the organic matter into the different sinks (Poni *et al.*, 2006). They also simulate disease severity as a proportion of the leaf area showing disease symptoms and having reduced photosynthetic activity. Working with the causal agent of leaf blast in rice, *Piricularia oryzae*, Bastiaans (1991) derived a model to relate the net photosynthetic rate of the leaf area with visible or 'visual' lesions to the photosynthesis of the leaf area without lesions. The model assumes that the visual lesion is part of a 'virtual lesion' that includes tissue without visible lesions but with reduced rates of photosynthesis. A ratio of virtual lesion area to visible lesion area (β in Bastiaans' model) > 1 indicates that photosynthesis is strongly inhibited beyond the margins of the visible lesion. Virtual lesions in downy mildew-infected grapevine leaves have been documented by Moriondo *et al.* (2005), but the size and physiological effects of virtual lesions have not been quantified.

In the current study, experiments were carried out to measure the gas exchange responses of grapevine leaf tissue to *P. viticola* infection during the asymptomatic stage of the infection (i.e. during the incubation period) and in the green tissue surrounding the visible downy mildew lesion.

Methods

The experiments used potted grapevine plants of the cultivar Barbera that were hedgerow trained and regularly watered. Leaves of different ages (i.e. different position on the shoot) were randomly selected and simultaneously inoculated on the abaxial leaf surface with a suspension of *P. viticola* sporangia that had been collected from naturally infected vineyards. Other leaves were not inoculated and served as healthy controls.

Chlorophyll concentration was determined using a SPAD 502 chlorophyll meter (Minolta Co., Japan). Net assimilation (A, $\mu mol \cdot CO_2/m^2/s$), transpiration (E, $\mu mol \cdot H_2O/m^2/s$), stomatal conductance (gs, $mmol/m^2/s$), intercellular CO_2 concentration (C_i, ppm), and difference between air and leaf surface temperatures (ΔT, °C) were measured in both inoculated and uninoculated leaf tissue by using a LCPro+ portable photosynthesis system (ADC Bioscentific Ltd., UK). These variables were measured from 11.00 am to 12.30 pm under saturating light intensity, i.e. photosynthetic active radiation (PAR) ≥ 900 $\mu mol/m^2/s$. The leaf area analysed was 6.25 cm^2, which was the size of the analyzer window.

In the first experiment, data were periodically collected from 1 day after inoculation to the day when sporangiophores first appeared on the leaves. Gas exchange measurements (A, E, gs, and C_i) in the inoculated leaves were expressed as a proportion of the values measured on healthy leaves of the same age (for instance, $A_{inoculated}/A_{uninoculated}$). For comparison of data collected with different replicate leaves, time was expressed in relation to the duration of the incubation period, i.e. time = 0 on the day of inoculation, time = 1 on the day when disease symptoms appeared, and time >1 on the day of sporulation.

In a second experiment, gas exchange was measured on affected leaves at different distances from the centre of the downy mildew lesion; the analyzer was used across the oil spot border with 50 and 25% of its area occupied by symptomatic tissue, or it was used on the symptomless tissue at increasing distances (from 1 to 4 cm) from the lesion border.

Results

The inoculated leaves had oil spot symptoms after 7 to 9 days of incubation; the pathogen sporulated 3 to 5 days later. The visible lesions ranged from 0.5 to 2.5 cm in diameter. The chlorophyll concentration in both affected and unaffected leaves did not significantly change during the infection cycle (data not shown). Similarly, the intercellular concentration of CO_2 did not significantly

differ between healthy and affected leaves (Figure 1a).
Net assimilation rate (A) was similar for the affected and healthy leaves until about 70% of the incubation had passed; afterward, A for affected leaves decreased until the beginning of sporulation (Figure 1b). Transpiration (E) and stomatal conductance (g_s) showed similar patterns (Figure 1c and 1d, respectively). The difference between air and leaf temperature progressively increased in the affected leaves starting from 70% of incubation because of stomata deregulation (Figure 2); just before symptom onset and at the beginning of sporulation, average ΔT was about 1.6°C higher in affected than in healthy leaves.

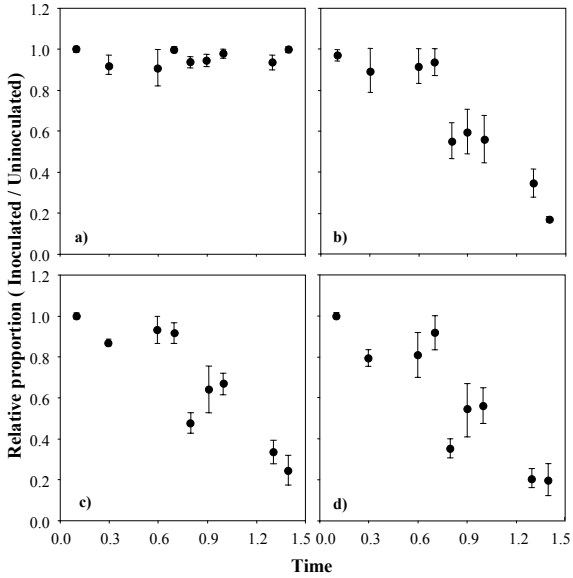

Figure 1: Changes in intercellular CO_2 (a), net assimilation (b), transpiration (c), and stomatal conductance (d) in grapevine leaves inoculated with *Plasmopara viticola* during the infection cycle. Data are expressed as a proportion of the values of same-aged healthy leaves; time is expressed in relation to the duration of the incubation period: time = 0 on the day of inoculation, = 1 on the day of disease symptom appearance, and >1 after symptom onset and until sporulation.

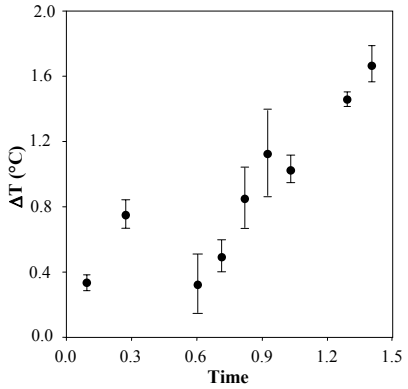

Figure 2: Differences between leaf and air temperature on grapevine leaves inoculated with *P. viticola*. Time is expressed in relation to the duration of the *P. viticola* infection cycle: 1.0 is the time of symptom appearance and 1.4 is the onset of sporulation.

The effect of *P. viticola* infection on gas exchange traits was also influenced by the distance from the infection site (Figure 3). An approximately 2-cm zone of the green tissue surrounding the oil spot showed a reduced photosynthetic capacity compared to the healthy tissue (Figure 3).

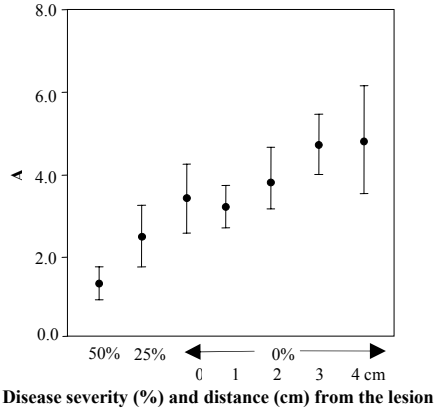

Figure 3: Net assimilation rate measured on the downy mildew lesion (the analyzer sensor covered 50% and 25% of the area occupied by the lesion) and on green tissue at different distances from the lesion. The average A measured on same-aged healthy leaves during the same period was 11.9 ± 0.51 μmol·CO_2/m^2/s.

Photosynthesis data were then analysed in order to relate visible lesions with virtual lesions and the relative photosynthesis rate. According to Bastiaans (1991), the fraction of leaf area remaining virtually healthy (1–y) can be estimated as:

$$(1 - y) = (1 - \beta\alpha)^n$$

where y is the fraction of leaf area that is virtually diseased, β is the ratio between the leaf area occupied by the virtual lesion and the leaf area occupied by the visual lesion, α is the fraction of the leaf area occupied by a single lesion, and n number of lesions per leaf.
The relative photosynthesis of an infected leaf is expressed as a function of disease severity through the equation:

$$P_x / P_o = (1 - x)^{\beta}$$

where P_x is the photosynthesis of a leaf with disease severity x, P_o is the photosynthesis of a healthy leaf, and x represents the fraction of total leaf area with visible disease.
Forty leaves were generated using the Assess software (APS, St. Paul MN USA) with severity of the visible lesions ranging between 0 and 1 (i.e. 100% of leaf tissue affected); size and position of the lesions on the leaf blade were generated randomly. Size of the virtual lesions was determined using data from Figure 3. Assimilation rate of these leaves was also calculated using the data of Figure 3 and expressed as a relative rate compared to the unaffected leaves. The parameter β was then calculated as indicated by Bastiaans (1991) for the relationship between visible and virtual disease severity (Figure 4a) and between visible disease severity and relative assimilation rate (Figure 4b). β was 9.0 for the relationship between visible and virtual lesions (Figure 4a) and 4.5 for the relationship between visible disease severity and relative assimilation rate (Figure 4b).

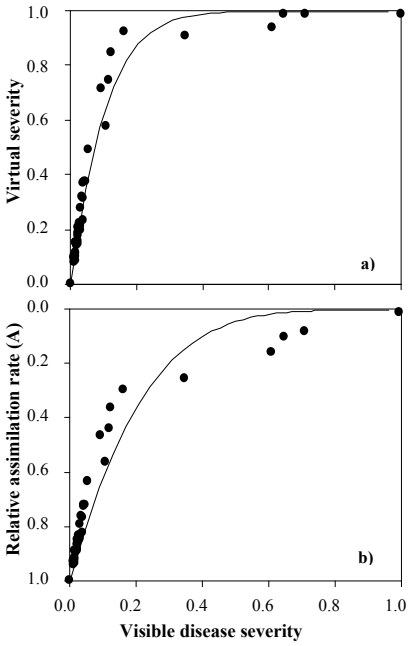

Figure 4: Relationship between visible disease severity (expressed as the ratio between visible symptomatic area and total leaf area) and virtual severity (a) and relative assimilation rate (b).

Discussion

This work confirmed the impact of *P. viticola* infection on grapevine leaf photosynthesis and the consequent reduction in carbon uptake and biomass accumulation. This work produced two main outcomes. First, reductions in photosynthesis occur before the appearance of visible lesions even though the chlorophyll concentration of affected leaves is similar to that of the healthy ones.

Second, photosynthesis is also reduced in the green tissue surrounding the downy mildew lesions. This finding confirms the hypothesis that the pathogen reduces leaf assimilation and transpiration in an area larger than that occupied by visible lesions (Bastiaans, 1991; Moriondo *et al.*, 2005). Unlike leaf rust on wheat and other host–pathogen systems in which the value of β is not significantly different from 1, i.e. host–pathogen systems in which the photosynthetic activity of the green area of the infected leaf is not affected (Spitters *et al.*, 1990), downy mildew on grapevine affects photosynthesis beyond the area of the lesions. The value of β of Bastiaans (1991) clearly expresses the relation between visible severity (area covered by oil spots) and virtual severity (total area affected by the pathogen). The value of $\beta = 9.0$ found in this work is similar to that ($\beta = 8.74$) found for powdery mildew on wheat (Rabbinge *et al.*, 1985). Nevertheless, the same value of β does not accurately characterize the effect of the pathogen on photosynthesis because it assumes that little or no photosynthesis occurs in the virtual lesion.

A lower value ($\beta = 4.5$) described this trait more precisely, meaning that the virtual lesion maintains a residual photosynthetic rate that is far from being negligible.

In conclusion, the current study indicates that models simulating the detrimental effect of downy mildew infection on photosynthesis of grapevine leaves must account for the reductions occurring during the incubation period of the *P. viticola* lesions and in the asymptomatic leaf tissue surrounding the visible lesions.

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Downy mildew control based on the plant physiology

M. Jermini[a], Ph. Blaise[b], C. Gessler[b]

[a] Research station Agroscope Changins – Wädenswil ACW, Centre of Cadenazzo, CH-6594 Contone, Switzerland; [b] Plant Pathology, Institute of Integrative Biology, Department of Agronomy, Swiss Federal Institute of Technology, Universitätstrasse 2, 8092 ETH-Zürich, Switzerland

Introduction

Today, it is exceptional to find important yield quantity losses caused by downy mildew epidemics in commercial vineyards; it is more common to observe epidemics causing different levels of leaf damage, which affect plant growth and yield quality. This is the result of the progress made in fungicide development, which has improved the downy mildew control especially with respect to the current requirement of maximal quality-quantity yield over a long period. In this crop system concept, the current pest or disease control strategies employed consider the plant as the growing substrate necessary to the fulfilment of their crop production cycle. This has led to a linear conception of the relation between quantitative presence of the pest or diseases, time and final damage with a partial analysis of the potentially real causes of the observed damages. However it is plausible and evident that this relationship is considerably altered by various host related factors that are influenced by growth stage and the environment. Consequently, our concept of control strategies underestimates the interactions with the crop system. To improve our IPM strategies it is therefore necessary to change this concept. The plant should be considered the centre of the crop system and the disease, or the pest, a stress factor for the plant, where the quantification of their interactions should be the basis upon which to implement a new disease management system. This analysis has been carried out for the downy mildew, quantifying: 1) the damage (impact on leaf gas exchange, plant growth and yield); 2) the plant compensation capacities; 3) the plant recovery capacities; and 4) the impact of the developed control strategy on the downy mildew epidemic, on yield and plant growth as well as on the recovering capacity of the plant.

Material and methods

The experiments were carried out in a vineyard of the research station Agroscope Changins-Wädenswil ACW Centre of Cadenazzo planted with the cv. Merlot grafted on 3309 rootstock. The vines were double cane pruned and vertical trained (double Guyot.).

The damage and compensation analysis as well as the recovering capacities were studied during the period 1995-1998 comparing three different treatments: A) "Untreated canopy" (to prevent quantity losses, the clusters were treated once with a contact fungicide at the discovery of the first downy mildew sporulation); B) "Reduced fungicide schedule" (based on a first treatment at the appearance of the first symptoms, to avoid yield quantity losses followed by one or two additional fungicide applications during the early epidemic phase with the aim of delaying the epidemic), and C) "Standard schedule" (schedule normally applied in the vineyard). The experimental plots were moved each year to avoid stress influence due to a repetition of the trials on the same place.

The analysis of the impact of the developed control strategy, called Minimal Fungicide Strategy (MFS), was carried out in comparison with a standard schedule (SS) used in the vineyard. The MFS was continuously applied on the same plot. The efficacy of the MFS was evaluated during the period 1999-2002 and the recovery capacities of the plant during the period 2003-2004.

Results of the damage and compensation analysis

For the downy mildew impact on plant growth, yield quality and the plant compensation mechanisms, only the results 1998 are presented.

Impact on leaf gas exchange. A drastic reduction in the photosynthetic rate was observed on the sporulating area of main and lateral leaf tissues. Stomatal and mesophyll conductance decreased and stomatal resistance increased, indicating the difficulty of CO_2 diffusing through the stomata into the mesophyll to the site of carboxylation. Downy mildew affected more negatively the gas exchange parameters on the symptomless parts of a diseased lateral than of a main leaf, indicating a greater susceptibility of lateral leaves. A decrease of stomatal conductance and, consequently, of the photosynthetic rate (Figure 1), Figure 1: Relationship between disease severity and the relative photosynthesis rate expressed as ratio between values measured on symptomless area of infected and healthy leaves. Squares refer to main leaves and circles to lateral leaves.

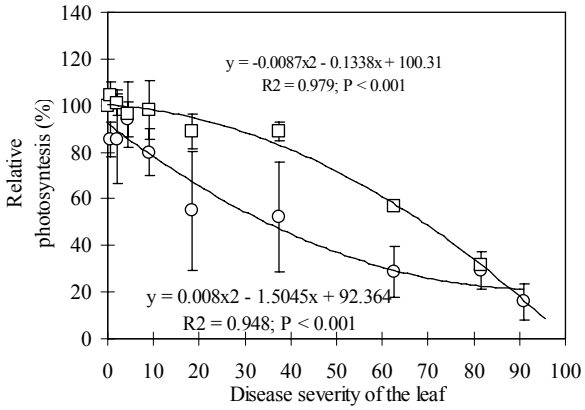

Figure 1: Relationship between disease severity and the relative photosynthesis rate expressed as ratio between values measured on symptomless area of infected and healthy leaves. Squares refer to main leaves and circles to lateral leaves.

transpiration and water use efficiency was observed even at low disease severity levels and the impact increased with severity on the leaf. At the same time an increase of stomatal resistance on the symptomless area was

measured. Visual assessment of the diseased leaf area didn't reflect the actual colonized area since at least a portion of the asymptomatic leaf area was in fact a latent lesion. Therefore, the visual estimation of downy mildew infection may not give a good indication of the effect of the pathogen on host physiology. These results also emphasized the important role of downy mildew as a stress element for the plant during ripening phase.

Impact on plant growth and yield quality. The epidemic progress in the treatment A showed an increase in disease severity starting from the beginning of the ripening phase (Figure 2A). Disease did not influence the amount of total healthy leaf area per plant until veraison (Julian day 219). From this phenological stage until harvest, the healthy leaf area per plant decreased rapidly at the same time as the epidemic increased (Fig 2B). At harvest, the yield quantity was affected (not for the years 1996 and 1997) and the significant difference was probably due to an insufficient level of yield regulation in the "Standard schedule" plots and a higher berry weight. Generally, a single fungicide application at the discovery of the first sporulation was enough to preserve the crop production. Among the yield quality parameters, the sugar content was negatively influenced by the downy mildew leaf damage (Table 1).

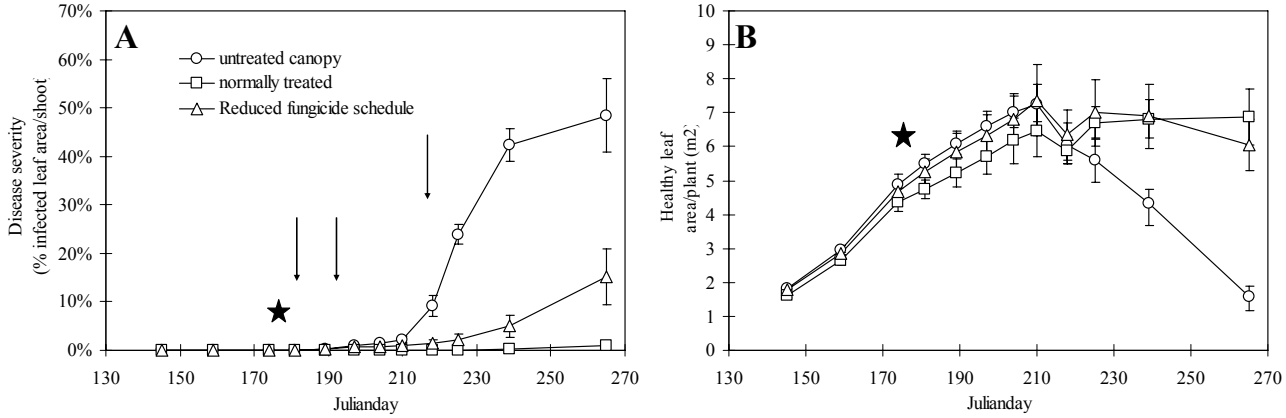

Figure 2: Disease severity progress as percentage of diseased leaf area per shoot (A) and evolution of the healthy leaf area/vine, expressed in m^2 per vine (B) in 1998. The star indicates the appearance of the first downy mildew sporulation in field and the arrow indicates the fungicide applications in the treatments "Untreated canopy" (only the first application on clusters) and "Reduced fungicide schedule"

Table 1: Effect of downy mildew epidemic on yield quantity and juice quality of Merlot grapevine at harvest for the experimental year 1998.

Attribute	Untreated canopy A	Reduced fungicide schedule B	Standard schedule C
Yield (kg/m^2)	1.022 b	1.105 b	1.357 a
Berry weight (g)	1.72 b	1.91b	1.97 a
Soluble solids (°Brix)	17.4 b	19.1 a	19.4 a
Juice pH	3.58 a	3.40 b	3.44 c
Titratable acidity (g/L)	5.46 b	5.92 a	5.56 b

The difference was particularly evident between the treatments A and C, but no differences were observed between treatments B and C. Disease severity limited between 1% at the beginning of veraison and 5% at the end of August, corresponding to the end of the first ripening phase, permitted the plant to compensate the stress situation without inducing a different sugar uptake in the berries during the ripening phase. No correlation between disease severity progress on canopy and sugar accumulation in the berries from veraison until harvest was found (Figure 3), indicating the capacity of the vine to compensate a stress situation induced by the downy mildew damage on leaf canopy.

Impact on the plant compensation mechanisms. The grapevine compensated for the carbohydrate requirements of the cluster by mobilizing the starch reserves stored in the woody parts and particularly in the roots. The defoliation level therefore has a central role. Consequently, treatment B provided an intermediary response for the carbohydrate requirement of the berries (Figure 4) and this is in agreement with experiments considering different artificial defoliations or crop load levels. Grapevines respond to a defoliation stress by altering the natural translocation pattern and directing carbon stored in the lower parts of the plant to the fruit in order to supply the assimilate requirements of the berries, which represent a very powerful sink during ripening. This compensation mechanism was applied without completely exhausting the reserves of each woody part and it suggested a hierarchical pattern of the mobilisation; first a mobilisation of the reserves stored in the roots, and secondly those from the trunk, cane and shoot.

Impact on the plant recovery capacities. The impact of the reserves reduction on the growth and fertility and the recovering capacity of the plant were analysed during two consecutive two-year periods (first year = stress; second year = recovering). Treatments A and C were compared. The impact of decreased reserve contents in the following growth season negatively influenced only the shoot elongation and the potential crop yield quantity of treatment A. Nevertheless, a single recovery year was enough to build up the reserve pool and particularly in the roots, confirming the acclimation potential of the grapevine.

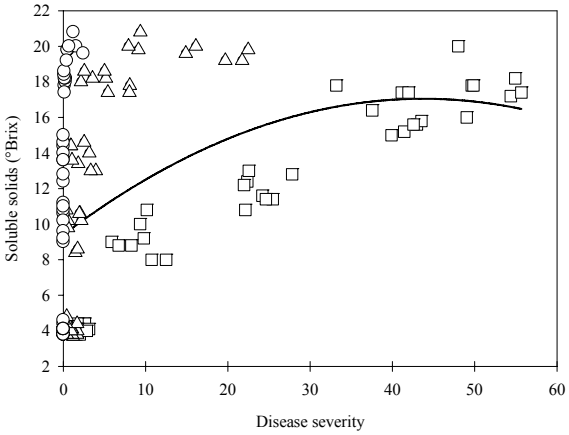

Figure 3: Relation between the must soluble solids and disease severity of *Plasmopara viticola* measured weekly from veraison to harvest for 1998. Squares correspond to "Untreated canopy", triangles to "Reduced fungicide schedule" and circles to "Standard schedule" treatments.

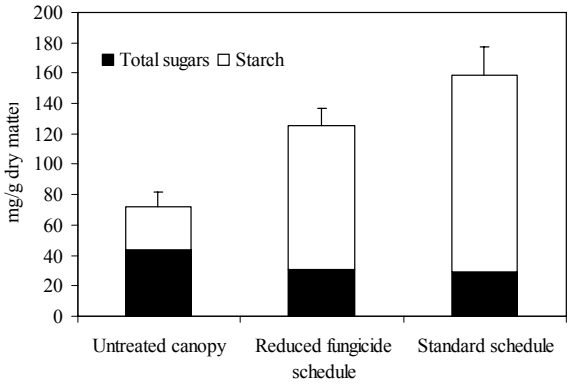

Figure 4: Effect of the downy mildew epidemics 1998 on the starch and sugar (glucose, fructose and saccharose) content expressed as mg for g dry matter (DM) of the roots.

Results of the Minimal Fungicide Strategy (MFS) application

The proposed MFS is based on a first treatment at the appearance of the first symptoms (to avoid yield quantity losses), followed by one or two additional treatments during the early epidemic phase to delay and keep the epidemic under a threshold that goes from 1% severity at the beginning of the veraison to 5% severity at the end of the first ripening phase (end of August). MFS aims to limit the plant stress during the ripening and therefore yield quality losses.

Efficacy of the MFS and effect of the cumulative stress on plant. MFS resulted in a reduction of the fungicide applications to between 43% and 66% and delaying the epidemic. On average, a decrease of 17% of the yield quantity was observed in the MFS plot, but the berry weight was generally higher than in the SS (Table 2). We can only suppose that these differences are due to the difficulty of regulating the crop load to a maximum of 1 kg/m^2 (production limit applied in Ticino) by eliminating the appropriate number of clusters/plant which in the MFS plot leads to an overly severe reduction. Nevertheless, the yield quantity obtained in the MFS plot can be considered appropriate.

Table 2: Results of the most important yield component at harvest from MFS and SS plots. Data are the average of the experimental years 1999-2002.

Attribute	Yield (kg/m^2)	Soluble solids (°Brix)	Titratable acidity (g/L)
SS	1.09 ± 0.17	19.60 ± 0.40	6.09 ± 0.887
MFS	0.91 ± 0.16	19.13 ± 0.71	6.51 ± 0.852

The same discussion is valid for yield quality, because the must soluble solids were generally lower at harvest in the MFS than in the SS, but the reduction was only 2.6% on average (Table 2). Although this limited number of fungicide applications delayed the epidemic progress, it was not possible to estimate in time the disease progress, and this remains an important limit. Consequently we were not able to contain the epidemic each year within the expected range of 1% to 5% at the beginning of the ripening phase. This was particularly evident in 1999 (first application year of the MFS), where the choice to begin leaf protection at a severity of 1.48% caused the most important losses at harvest. Nevertheless, the first two fungicide applications upon observance of the first sporulation played a central role because they limited the appearance of fit genotypes, which are generally responsible for the disease spread in the plot. Each growing season was independent of the previous one, because the reconstitution or depletion of the reserve pool depends on the plant stress during the ripening period. The impact of a repeated stress does not have important consequences on the plant growth. Only shoot elongation, from the third stress year and the total number of leaves per shoot in the fourth stress year were influenced. The potential yield quantity, estimated before crop regulation, was negatively influenced by repeated stress situations. However its consequences are negligible because the estimated production was always higher than the production limit applied in Ticino.

Recovering capacity of grapevine after accumulated stress years. A single recovery year (2003) without a stress situation was enough to eliminate the stress symptoms on plant created in the prior year, and this permitted the vine to return the carbohydrate reserves to a normal level for the following growing season (2004).

Conclusions

MFS applications delay the start of downy mildew epidemics, and are therefore sufficient to reduce the reserve mobilisation to a level that does not induce important depletion of the carbohydrate reserves. The impact of the downy mildew stress caused by the incomplete control of *P. viticola* on plant growth and yield quantity and quality is small. The risk due to the application of the MFS over several years is minimal. The decision for the fungicide application is the critical point. The optimal application of MFS necessitates quantifying the role of primary and secondary infection in the epidemic development. These factors should be implemented into a quantitative forecasting model that integrates, in addition to the epidemic development, the yield formation and their interactions with the disease.

Invasion history of grapevine downy mildew (*Plasmopara viticola*): a population genetic perspective

F. Delmotte[a], G. Louvet[a], S. Richard-Cervera[a], P. Mestre[b], A. Schilder[c], F. Austerlitz[d], M. C. Fontaine[d]

[a]INRA, UMR 1065 SV, Institut des Sciences de la Vigne et du Vin, Centre de Recherches Bordeaux-Aquitaine, BP 81, 33883 Villenave d'Ornon cedex, France (delmotte@bordeaux.inra.fr), [b]INRA, UMR 1131 SVQV, 28 rue de Herrlisheim, BP 20507, 68021 Colmar cedex, France, [c]Michigan State University, Plant Pathology Department, 181 Wilson Road East Lansing, MI 48824, USA, [d]ESE - Ecologie, Evolution et Systématique, CNRS/Université Paris-Sud XI, Bâtiment 360, 91405 Orsay Cedex, France

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Grapevine (*Vitis vinifera*, L.) is cultivated worldwide mainly for the production of wine, juice and fresh fruits. Viticulture is threatened by numerous pathogens, most of them being introduced by human activities. The Oomycete *Plasmopara viticola* (Berkl. and Curt.) Berl. and de Toni., causal agent of grapevine downy mildew, is an obligate biotrophic pathogen that attacks numerous species of the *Vitaceae*. *P. viticola* is a native species from North America that was introduced accidently into Europe in the late 1870s. This introduction was an indirect consequence of the previous invasion by the devastating root-feeding aphid phylloxera, which imposed the grafting of European varieties on to phylloxera-resistant rootstocks imported from North America that probably contained downy mildew inoculums. The disease spread rapidly through most of continental Europe to become the most important vineyard diseases. Since then, grapevine downy mildew has expanded all over wine producing regions worldwide: it was officially reported for the first time in 1907 in the Eastern Cape Province of South Africa and in South America, in 1917 in Australia, and in 1926 in New Zealand.

We assessed the genealogical history of *P. viticola* using seven microsatellite markers, partial sequences from two nuclear genes (*β*-tubulin, 28S) and a mitochondrial gene (cyt*b*) from more than 1000 isolates collected in North American, European (France, Spain, Italy, Germany, Switzerland, Austria, Hungary, Czech Republic, Romania, Greece) as well as in Australian and South African vineyards. Using population genetics and phylogenetics tools, we addressed the following questions related to the invasion history of *P. viticola* worldwide: what is the level of genetic diversity of grapevine downy mildew in its native range, i.e North America? From which populations in this native range originate the inoculums of *P. viticola* that were introduced in France in 1878? How many independent introductions occurred in Europe and is there a colonization gradient into the European vineyards? Did the subsequent introductions into new world wine producing areas (in particular in Australia and South Africa) result from a primary introduction from North America or a secondary introduction from European *P. viticola* populations?

Can early population structure of *Erysiphe necator* inform about the disease level on bunches?

P. Cartolaro[a], J. Montarry[ab], S. Richard-Cervera[a], F. Delmotte[a]

[a]INRA Bordeaux, UMR 1065 SV, Institut des Sciences de la Vigne et du Vin, BP 81, 33883 Villenave d'Ornon cedex, France (delmotte@bordeaux.inra.fr); [b]INRA Avignon, UR 407, Pathologie Végétale, 84140 Montfavet, France

Grapevine powdery mildew, caused by the biotrophic ascomycete *Erysiphe necator* (syn. *Uncinula necator*), is one example of a plant pathogen showing two genetically differentiated groups of isolates coexisting on the same host, *Vitis vinifera* (Délye *et al.* 1997; Evans *et al.* 1997; Miazzi *et al.* 2003; Nuñez *et al.* 2006). Several studies have suggested that genetic *E. necator* groups (A and B) correlated with ecological features of the pathogen; Délye *et al.* (1997) proposed that group A isolates over-winter as resting mycelium within dormant buds that reinitiate growth after budbreak and colonise young flag-shoots, while group B isolates would survive as ascospores released from overwintering cleistothecia. Indeed, an association between flag-shoot symptoms and infection by group A isolates has been found in earlier studies in France (Délye and Corio-Costet 1998; Amrani and Corio-Costet 2006) and Italy (Miazzi *et al.* 2003). Due to this association, these authors proposed that group A isolates may be responsible for early infections in the season while group B isolates may be responsible for late infections (Délye and Corio-Costet 1998; Miazzi *et al.* 2003). However, the association between genetic groups and over-wintering survival has been challenged by recent studies reporting that flag-shoot symptoms may harbour both group A and B isolates (Cortesi *et al.* 2005; Nuñez *et al.* 2006; Péros *et al.* 2005, Montarry *et al.* 2008). Moreover, the hypothesis of a temporal succession of genetic groups was based on genetic studies that suffered from sampling strategies confounding time during the epidemic with over-wintering mode and source of inoculum. Data available showed that the frequencies of the groups could vary greatly from one field to another, suggesting a high level of spatial heterogeneity at the vineyard scale (Cortesi *et al.* 2005; Amrani and Corio-Costet 2006; Montarry *et al.* 2008).

Here, our aim is to study the regional dynamics of *E. necator* genetic groups at a large spatial scale. We conducted a landscape genetic approach combining landscape epidemiology and population genetics in order to explore the geographic distribution of *E. necator* genetic groups in southern France vineyards, and to assess the temporal succession of groups along the course of the epidemics. Moreover, we have evaluated the relationship between the frequency of genetic groups and disease level on leaves and clusters at the end of the epidemics.

This study therefore addressed three questions: (1) what is the genetic variability (A or B) of *E. necator* populations on flag-shoots at a regional scale? (2) are there changes in the frequency of genetic groups between the start and the end of the epidemic? and, (3) is there a relationship between the frequency of genetic groups assessed early in the season and disease levels at the end of the growing season?

Material and Methods

Diseased leaves of cv. Carignan (*Vitis vinifera*) were randomly sampled twice during the 2007 growing season in commercial vineyards of the Languedoc-Roussillon region. The first sampling was performed in 32 vineyards early in the growing season (end of April) and the second sampling in 16 of those 32 vineyards at the end of the growing season (early September).

At the first sampling, diseased leaves were collected only on flag-shoots; at the second sampling, diseased leaves were randomly collected within each vineyard. This led to a total of 1,253 leaves infected with *E. necator* , of which 769 were sampled in April and 484 in September.

Molecular characterisation. The molecular method used to differentiate genetic groups was the amplification of the β-tubulin gene of *E. necator* (tub2, accession number AY074934) exhibiting a T/C single nucleotide polymorphism (SNP) between group A and group B isolates (Amrani and Corio-Costet 2006). SNP creates a recognition site of restriction endonuclease AccI that allows the characterisation of A or B isolates by Cleaved Amplified Polymorphic Sequence (CAPS) analysis (e.g. Baudoin *et al.* 2008, Montarry *et al.* 2008, Montarry *et al.* 2009).

Disease assessment on leaves and clusters. At the end of the 2007 growing season, prior to the grape harvest (mid-September), the disease levels on leaves and clusters were visually estimated in 13 out of the 32 vineyards sampled at the beginning of the epidemic for genetic analysis. That estimation, based on the observation of five areas (composed at least of 100 vines) randomly distributed in the field, took into account incidence of diseased vines (i.e. an estimation of the percentage of diseased vines) and global symptom severity (i.e. an estimation of the percentage of leaf area infected), using the following category scale: 0 = severity <5% and incidence 0–5%; 1 = severity <5% and incidence 5–20%; 2 = severity <5% and incidence 20–50%; 3 = severity <5% and incidence >50%; 4 = severity 5–30% and incidence >50%; 5 = severity >30% and incidence >50%.

Results

From the 769 lesions sampled at the beginning of the season, 659 (85.7%) yielded a PCR amplicon of the β-tubulin gene; from the 484 lesions sampled at the end of the season, only 205 (42.4%) did so. Among the 659 *E. necator* isolates collected at the beginning of the season from flag shoots, 440 (67%) belonged to group A and 219 (33%) to group B. This confirmed that both group A and group B isolates can over-winter as resting mycelium within dormant buds and lead to flag-shoot symptoms. The frequencies of the genetic groups per field varied greatly, from 100% group A (in ten fields) to 100% group B (in four fields). From the 18 fields showing a mix of A

and B isolates, ten contained a majority of group A isolates and eight a majority of group B isolates (Figure 1).

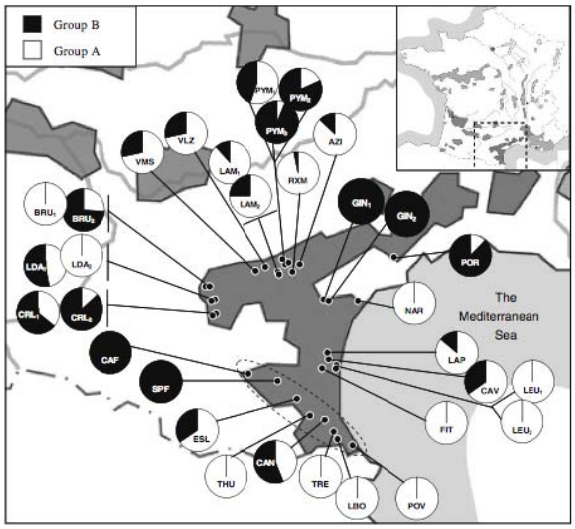

Figure 1: Spatial distribution of the 32 fields sampled in the south of France, and frequency of *Erysiphe necator* isolates belonging to group A (white) and B (black) for each field. The dotted line shows the geographical position of the Agly's Valley.

All isolates collected at the end of the growing season belonged to group B, whatever the initial frequencies of group A; thus, even populations composed of 100% A at the start (BRU1, FIT, LDA2, POV, THU and TRE) were 100% B at the end of the epidemic (Figure 2).
A strong relationship was observed between the disease levels on leaves and clusters, estimated at the end of the growing season in 13 fields, and the initial frequency of genetic group B (Spearman's rank correlation rho = 0.905, $P < 0.001$ for damage on clusters and Spearman's correlation rho = 0.756, P= 0.003 for damage on leaves). Every vineyard from which only B isolates were detected at the onset of the epidemic had a high final severity of disease (disease score >2); whereas vineyards infected by *E. necator* populations including group A isolates (from 26.1% to 100%) had a low final disease severity (disease scores 0 or 1) (Figure 3).

Discussion

The spatial genetic analysis of flag-shoot symptoms sampled early in the season revealed the absence of aggregation of genetic groups at the vineyard scale in southern France. This result indicates that a genetic group was not more likely to occur in a vineyard if it was close to other fields including *E. necator* populations of the same group. At the spatial scale studied here, the two genetic groups of *E. necator* appeared randomly distributed, and neither the altitude nor the distance to the sea correlated with their spatial distribution. These results confirm previous data showing inter-vineyard heterogeneity in southern France on a smaller number of populations (Amrani and Corio-Costet 2006), and invalidate the hypothesis of a niche partitioning due to a geographic separation of the groups.

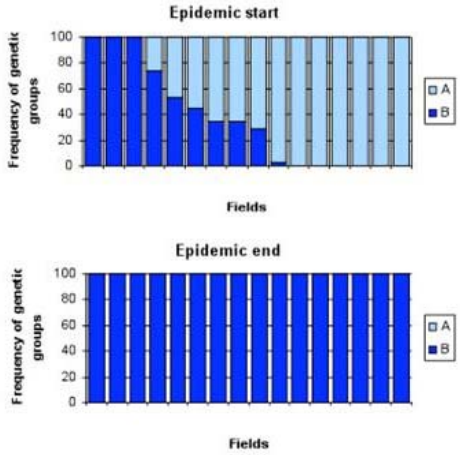

Figure 2: Temporal distribution of genetic groups of *E. necator* in the 16 fields sampled in the south of France.

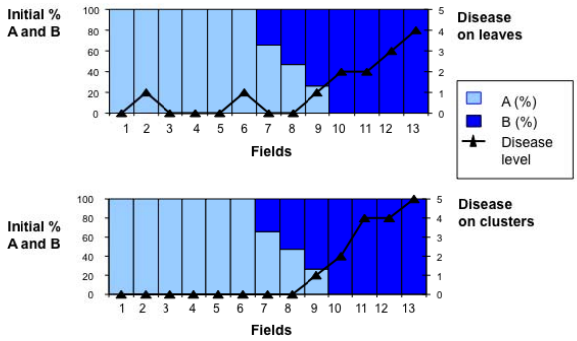

Figure 3: the disease levels on leaves and clusters, estimated at the end of the growing season in 13 fields, and the initial frequency of *E. necator* genetic group B.

While the spatial distribution of genetic groups of *E. necator* proved to be random, the temporal dynamics of groups was the same in all fields: the monitoring of 16 different fields showed that group A isolates were active only at the beginning of the growing season and disappeared during the course of the epidemic; by contrast group B isolates are active during the entire epidemic and were responsible for late infections. Our results support a temporal differentiation of niches of the two groups.
Since results obtained from different regions and different years have evidenced the decline of group A isolates during the course of the epidemics, our finding might not be due to environmental conditions specific for the 2007 growing season. Moreover, our results based on a set of 659 genotyped isolates collected from flagshoot symptoms, confirmed that both genetic groups are able to overwinter asexually in buds (Cortesi *et al.* 2005; Péros *et al.* 2005; Nuñez *et al.* 2006). Our data suggest that only B isolates could produce cleistothecia via sexual reproduction in vineyards, which takes place at the end of the growing season.
Our data showed that damage due to *E. necator* on leaves and clusters was less important in commercial vineyards where epidemics started with populations including A isolates than in vineyards showing flagshoot symptoms caused by group B isolates. The strong association between disease severity at the end of the growing season

and the initial composition of the populations raises new questions with both practical and theoretical interests. A hypothesis to explain the association between the initial frequency of A and B groups and damage on clusters at harvest could lie in a difference in aggressiveness on berries between *E. necator* genetic groups. Because the susceptibility period of clusters is restricted to about two weeks after bloom, and assuming a higher aggressiveness of B isolates on berries, the genetic composition of *E. necator* populations during the susceptibility period of clusters could be the major factor driving damage on berries at harvest. Thus, an initial attack by a population mainly composed of group B isolates (aggressive on berries) would cause severe damage at harvest; conversely, if group B isolates increase in frequency only later (i.e., when the ontogenic, or age-related resistance of clusters is active) then the epidemic will cause little or no damage at harvest.

Interestingly, the ontogenic resistance of leaves is less limited in time because of the continuous growth of the vine. This might explain why the association between the frequency of genetic groups and disease levels was slightly stronger on clusters than on leaves. In order to test our hypothesis, further experiments are needed to investigate the aggressiveness of each *E. necator* genetic group on leaves and berries. Moreover, because our observations were based on a limited number of vineyards/populations and did not take into account chemical protection, it will be necessary to follow the epidemic development on leaves and clusters in crops showing different frequencies of genetic *E. necator* groups and with standardised farming methods. A landscape genetic approach will help to determine ecological factors involved in the temporal and spatial genetic variability of *E. necator* populations. The identification of factors favouring one group over another will provide useful information for an integrated crop management with limited fungicide use.

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Session 2: Poster

Diversity and Fitness of *Plasmopara viticola* isolates resistant to QoI fungicides

M. F. Corio-Costet[a], M. C. Dufour[a], J. Cigna[a], P. Abadie[b], and W. J. Chen[c]

[a] INRA, UMR Santé Végétale 1065 (INRA-ENITA), BP 81, F-33883 Villenave d'Ornon, France, [b] INRA, UMR Biogeco, 33612, Cestas, France, [c] Institut of Oceanography, National Taiwan University, Taipei, 10617, Taiwan

Effective control measures for downy mildew caused by the oomycete *Plasmopara viticola* include Quinone outside inhibitor fungicides (QoIs). However, strong selection pressure following repeated QoI applications may result in the development of QoI-resistant populations, and this has limited the efficacy of these fungicides in grapevine (Grasso *et al.*, 2006). Only a few years after the introduction of QoI fungicides, *P. viticola*-resistant populations were detected throughout European and United States vineyards (Baudoin *et al.*, 2008; Corio-Costet *et al.*, 2008; Sierotzki *et al.*, 2005).

In most pathogens, two major mutations in the cytochrome b gene have been reported (G143A, F129L) to be involved in resistance mechanisms, with the G143A mutation most widely recognized (Gisi *et al.*, 2002). Chen *et al.* (2007) investigated the mechanism underlying the evolution of QoI resistance, by carrying out a phylogenetic analysis of a large mitochondrial DNA fragment including the cytochrome b gene (2.281 bp) across a wide range of *P. viticola* isolates. Four major haplotypes belonging to two distinct genetic groups (I and II) were identified, each of which contained a different QoI fungicide-resistance allele carrying the G143A mutation (Chen *et al.*, 2007). These findings thus indicated that there were at least two origins of fungicide resistance in grapevine downy mildew populations. In France, group I (IR and IS) and group II (IIR and IIS) haplotypes have reached a mean frequency of 75% and 25% respectively, but their distribution may differ between regions (Corio-Costet *et al.*, 2008; Chen *et al.*, 2007). The resistant haplotype IR has been found to account for 67.7 to 98.3% of the resistance alleles in the population, depending on the site considered (Chen *et al.*, 2007, Corio-Costet *et al.*, 2006).

One challenge facing researchers trying to understand the evolution of fungicide resistance is fitness measurements, assessing the selective value of the pathogen (Antonovics, and Alexander, 1989; Pringle and Taylor, 2002). The rapidity with which QoI resistance has appeared and the behavior of resistant isolates in populations suggest that the fitness of resistant isolates may be high. We assessed this here. We performed fitness and competitiveness studies to investigate the spread and maintenance of *P. viticola* and we assessed i) the diversity of QoI haplotypes in European countries, ii) the fitness of sensitive and resistant isolates by comparing latent period, sporulation, infection frequency and competitiveness and iii) the cost of QoI resistance.

Material and Methods

A total of 1366 downy mildew lesions on *Vitis vinifera* were collected between 2000 and 2004 (table 1). For isolates collection, a total of 11 QoI-sensitive isolates and 12 QoI-resistant isolates were collected mainly in 2003 at the beginning of the growing season on leaves in Bordeaux vineyard and one in Germany (table 2).

Table 1: Mean mitochondrial haplotype distribution in European populations of *Plasmopara viticola*

Country	N	% of European mitochondrial haplotypes			
		IS	IR	IIS	IIR
France	1015	56.45	20.99	20.3	2.26
Germany	93	73.12	8.6	18.28	0
Greece	21	90.47	0	9.53	0
Italy	41	85.37	0	14.63	0
Portugal	68	79.41	2.94	17.65	0
Romania	67	97.02	0	2.98	0
Switzerland	61	80.33	0	19.67	0
Europe (Mean ± Sem)	1366	**80.19 ± 4.98**	**4.14 ± 2.91**	**15.18 ± 2.56**	**0.5 ± 0.5**

To characterize the sensitivity of *P. viticola* isolate to QoI fungicides, single lesions were multiplied onto leaves of *Vitis vinifera.* Inoculation was performed as previously described (Chen *et al.*, 2007; Corio-Costet *et al.*, 2006). The QoI tested was famoxadone (3-anilino-5-methyl-5-(4-phenoxyphenyl)-1, 3-oxazolidine-2, 4-dione). A discriminatory dose of 10 mg/l, was used to determine whether a strain was resistant or sensitive. Ranges of concentrations (from 0 to 1000 mg/l for resistant strains and from 0 to 2 mg/l for sensitive strains) were used to determine the ED_{50} for each isolates. Fungicide assay were carried out as previously described (Corio-Costet *et al.*, 2008, Corio-Costet *et al.*, 2010).

We measured the aggressiveness components, by quantifying thee latent period, sporulation and infection frequency as previously described (Chen *et al.*, 2007), on three plates each, containing 5 leaf discs inoculated with three droplets of a suspension of 2500 sporangia/ml. The latent period (LP) was estimated by monitoring the daily development of sporangia and determining the mean time required to obtain 50% sporulation. Spore production (Nt/Nt0) was assessed by washing the five inoculated discs from each plate in a vial with Isoton solution and determining the number of sporangia produced per plate in a Coulter multisizer counter. Infection frequency (IF) was defined as the proportion of inoculated leaf discs on which lesions developed 7 days after inoculation. A composite fitness index (Fi) was then calculated from the formula $Fi = Ln (N_t / N_{t0} \times IF \times 1/LP)$ (Corio-Costet *et al.*, 2010).

The relative competitiveness of two pairs of resistant/sensitive isolates R1-S1 and R2-S1 was compared at three initial R:S ratios of spores concentrations: 20:80, 50:50 and 80:20. Mixed inocula were generated by mixing such that the final suspension contained 40,000 spores per ml. Four plates, each containing 5 discs, were inoculated. We then followed a series of eight consecutive asexual cycles. We quantified the proportion of resistant isolates after each asexual cycle, by carrying out a biological test with 100 mg/l famoxadone, as described above, and a QoI real time Q-PCR quantification with specific primers for

quantification of the *Cyt b* gene and the resistant allele described by Sirven *et al*, 2002 (Sirven *et al.*, 2002).

Results

The SNP typing assay was used to survey the geographical distribution of various mitochondrial haplotypes from a panel of 1366 *P. viticola* isolates. The frequency of the four main European haplotypes was estimated (Table 1). Haplotype group I (IS and IR) predominated in all countries, accounting 77.44 to 97.02% (mean 84.33%). Haplotype II (IIS and IIR) accounted for 2.98 to 22.56% depending on the countries, with mean 15.68%. Haplotype IR predominated among strains resistant to QoIs, as expected accounting for 0 to 20.99% (mean 4.14%)of strains. The resistant haplotype IIR was the least common.

We evaluated the sensitivity to QoIs of 11 isolates classified as sensitive and 12 isolates classified as resistant collected in 2003 (Table 2). Sensitive isolates had ED_{50} values of 0.1 to 0.9 mg/l whereas those for resistant isolates exceeded 1000 mg/l. Seven sensitive isolates belonged to haplotypes II, the remaining four belonging to haplotype I. By contrast, 8 resistant isolates belonged to haplotype I, whereas only 4 belonged to haplotype II. The mean relative resistance factor between resistant and sensitive was 5263.

Table 2: Origin and characteristics of monosporangial isolates used in fitness studies

Isolate[1]	Locality of vineyard	Year	Mitochondrial haplotype (I or I)	QoI sensitivity	QoI CI_{50} (mg/ml)
S1	Bordeaux	2003	I	S	0.07
S2	Champagne	2003	II	S	0.90
S3	Bourgogne	2003	II	S	0.10
S4	Bordeaux	2003	II	S	0.05
S5	Bordeaux	2003	II	S	0.10
S6	Bordeaux	2003	II	S	0.07
S7	Champagne	2003	I	S	0.22
S8	Bordeaux	2003	II	S	0.13
S9	Bourgogne	2003	II	S	0.04
S10	Rhône Valley	2003	I	S	0.09
S11	Alsace	2003	I	S	0.28
R1	Midi-pyrénées	2003	I	R	>1000
R2	Bordeaux	2003	II	R	>1000
R3	Bordeaux	2003	II	R	>1000
R4	Midi-pyrénées	2003	I	R	>1000
R5	Freiburg	2003	I	R	>1000
R6	Midi-pyrénées	2003	I	R	>1000
R7	Bordeaux	2003	I	R	>1000
R8	Bordeaux	2003	I	R	>1000
R9	Bordeaux	2003	I	R	>1000
R10	Bordeaux	2003	I	R	>1000
R11	Bordeaux	2003	II	R	>1000
R12	Bordeaux	2003	II	R	>1000

Data on the fitness of the 23 isolates inoculated separately (Table 3) showed there to be no difference (P= 0.523) in latent period between sensitive and resistant isolates. The latent period was between 86.5h to 140h for sensitive isolates and 89.5 to 139h for resistant isolates.

No significant difference in spore production was observed between sensitive and resistant isolates (P= 0.137). Sensitive isolates produced a mean of 704 ± 139 sporangia and resistant isolates produced 830 ± 171 sporangia per deposited sporangium (Table 3). Considerable variation was observed within the sensitive isolate group and the QoI-resistant group. In our experimental conditions, there was a significant difference in infection frequency between sensitive and resistant isolates with 0.84 ± 0.035% of sensitive isolates and 0.94 ±0.02% of resistant isolates successfully infecting leaves (P=0.02). IF was between 0.42 and 0.97 in the sensitive group and between 0.68 and 1 in the resistant group.

A composite fitness index was calculated for each isolates based on the F_i of Tooley (1986). Mean Fi was 1.75 ± 0.65 for sensitive group and 2.05 ± 0.45 for the resistant group, but this difference between the groups was not significant (P=0.147). The fitness index varied considerably depending on whether the isolates were sensitive or resistant. The Fi value obtained was then used in the selection of sensitive and resistant isolates for competitiveness tests.

Table 3: Fitness components of sensitive and QoI-resistant isolates on grapevine leaf disks at 22°C (means ± SEM).

Isolates	QoI Sensitivity	Latent Period (h ± SEM)	Sporulation [1] (Nt/Nt_0) (± SEM)	Infection Frequency (± SEM)	Fi (Ln (Nt/Nt_0 IF /LP)
11 sensitive	S	**102.7 ± 1.57**	**704 ± 139**	**0.84 ± 0.035**	**1.75** ± 0.65
12 resistant	R	**100.9 ± 1.32**	**830 ± 171**	**0.94 ± 0.02**	**2.05** ± 0.45

[1]: Sporulation is expressed as the ratio of spores produced per deposited spore after 7 days of growth ± SEM.

The objective was to assess the competitiveness of resistant and sensitive isolates in mixed-isolate inoculations on leaf disks and to link these findings with the fitness parameters of isolates. Changes in the frequency of resistant isolates were largely dependent on the competing isolates and on their initial fitness index. In figure 1, the sensitive isolate S1 had an Fi value of 1.78, a figure typical of sensitive isolates (mean 1.75, Table 3). The resistant isolate R1 with which it was mixed (figure 1) had an F_i of 2.15 close to the mean value for resistant isolates. In the second pair tested, we mixed an S1 with an R2 isolate (figure 1), which had an Fi (1.57) lower than the Fi of the S1 isolate. After eight asexual cycles all ratios between sensitive and resistant isolates remained stable at 20, 50, and 80% R1, after quantification by biological assays and Q-PCR. A small but significant increase (P< 0.05) from the initial ratios of 20% and 50% was observed in the eighth cycle. In the second pair (figure 2), a decline in the frequency of R2 was observed after five cycles, for all three initial ratios, indicating that the resistant isolate R2 competed only weakly with its S1 partner.

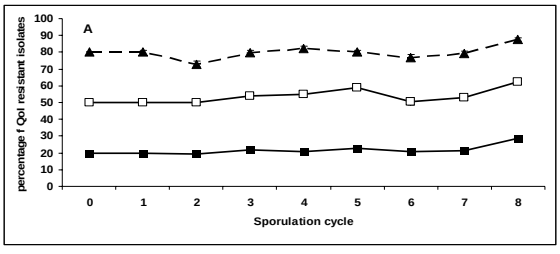

Figure 1: Dynamic changes (means ± SEM) in the frequency of QoI-resistant isolates in the sporangial populations harvested from grapevine leaf disks inoculated with three mixtures of QoI-sensitive S1 and QoI-resistant sporangia R1 in various proportions (20:80 (-■-), 50:50 (-□-) and 80:20 (--▲--), with monitoring over eight asexual generations. The second sporulation cycle was initiated with sporangia resulting from the initial infection and subsequent sporulation cycles were

initiated in a similar manner. Each point is mean of six replicates. **A**: assessment of QoI-resistant sporangia by biological tests

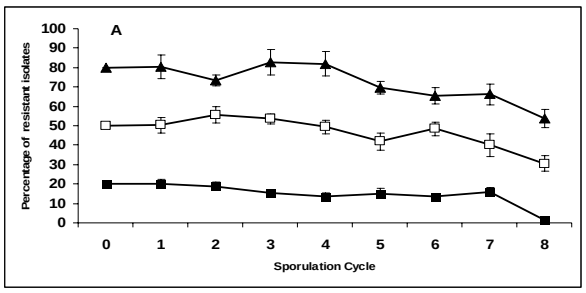

Figure 2: Dynamic changes (means ± SEM) in the frequency of QoI-resistant isolates in sporangial populations harvested from grapevine leaf disks inoculated with three mixtures of QoI-sensitive S1 and QoI-resistant sporangia R2 in various proportions (20:80 (-■-), 50:50 (-□-) and 80:20 (--▲--), over eight asexual generations.

Conclusion

Previous results for French vineyards (Corio-Costet *et al.*, 2008; Chen *et al.*, 2007, Corio-Costet *et al.*, 2006), showed haplotype1 to be the most widespread in Europe accounting for a mean of 84.33%. Only 4.54% of the 1366 isolates collected between 2000 and 2004 were QoI-resistant (IR, IIR), with haplotype IR accounting for 91.19% of resistant haplotypes. The role of mitochondrial haplotype I and II in the *P. viticola* population remains unclear, although it has been shown that QoI resistance in downy mildew in Europe has at least two different origins (6). It is clear that, at the beginning of the growing season, before fungicide selection pressure, most isolates are sensitive to QoI after the sexual reproduction cycle.

One of the most important factors affecting the evolution of fungicide resistance is the fitness of resistant isolates. In our study the fitness parameters of QoI-sensitive and resistant field isolates of *P. viticola* show that i) resistant isolates are as fit as sensitive isolates, in terms of sporangium production, latent period duration and infection frequency in asexual cycles, ii) QoI resistant isolates may compete successfully compete with sensitive isolates in mixed inoculation if their fitness index is sufficiently high.

No cost of QoI resistance was detected in our experimental conditions. There may be three reasons for this i) QoI resistance due to a single mutation (G143A) may have no cost, ii) there may be a cost but selection acts on resistant isolates that initially have a very high fitness level, so the cost is not detectable, or iii) there is a cost, but additional compensatory mutations or regulations restore the fitness of resistant strains.

In the case of QoI resistance, mitochondrial heritability and the frequency of sexual reproduction also determine the likelihood of the resistant allele spreading. In agriculture, a better understanding of fungal evolution might improve active manipulation of the evolutionary process, making it possible to reduce fungicide resistance in natural *P. viticola* populations.

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Genetic variability in populations of *Erysiphe necator* in Israel

T. Zahavi[a], R. Cohen[b], N. Katzir[b], G. Sapir[c], M. Reuveni[d]

[a]Extension service, Ministry of Agriculture, Israel, [b]ARO, Volcani center , [c]Northern R & D, Kiriat Shmone. [d]Golan Research Institute, Univ. of Haifa, Katzrin, Israel

Powdery mildew (PM) caused by *Erysiphe necator* (Schw.) Burr, is a widespread and destructive disease of field-grown grapevines. The fungus can attack and develop on all green parts of the vine, may affect leaves, shoots fruits and rachis, and cause considerable crop loss. Survival of the fungus between seasons is believed to be as mycelia in the dormant buds or as cleistothecia. In warm regions, where the timing of the vine phonological stages is determined by agrotechnical measures as pruning and irrigation, green leaves can always be found and PM can continue its development year round. Earlier work in Israel, looking a PM epidemiology (Ovadia, Ph. D. thesis) concluded that cleistotecia are the main source of inoculum. The aim of the present study was to look at the structure of PM population in Israel using RAPD markers.

Molecular methods have been used to compare the genetic variability of PM isolates in order to understand the relative importance of the different means of survival (Delye *et al.*, 1997, Miazzi *et al.*, 2003 and Evans *et al.*, 1996). Our study examined PM isolates from different viticultural regions of Israel.

Twenty one and fifty five isolates were collected in 2006 and 2007, respectively from 5 growing regions (Figure 1). Single spores of each isolate were used to inoculate leaves in the lab and DNA was extracted from conidia and mycelium of the developing colony using MasterPureTM Yeast DNA Purification Kit (Epicentre, Madison, WI USA). All isolates were amplified with four RAPD primers: E7, P6, J5 and P19 (Operon inc.) generating a total of 67 amplicons. A data set was constructed consisting of all the arbitrary labeled fragments and their presence or absence in each isolate. Nei's resembling function (Nei and Li 1985) was used to classify and separate clusters of strains with different degrees of similarity. Results are expressed as a dendrogram, using similarity values to create clusters of strains.

The dendrogram shows five groups of isolates. The six flag shoot isolates were collected in three Carignan vineyards (2 isolates from each), from the same growing region. Though they grouped together they are not identical. Six additional isolates grouped with the flag-shoots isolates. These isolates were collected later in the season, four from Cabernet sauvignon and two from Superior seedless from two different growing regions. Most (16/18) of the isolates that were collected during the season in vineyards in the central part of the country belong to two groups, while 17 of 25 isolates from the northern part of the country belong to two other groups. The two isolates from the "Jordan Valley" region, a very hot and dry area, formed a separate group.

This work is the first and only one looking at the molecular structure of Israeli PM populations. The diversity found is higher then that reported from other countries. In our work, even flag shoots isolates that originated from the same vineyard were not identical as opposed to the findings of Delve (1997). Israel is a relatively hot country and rains usually stop around April, less then a month after the bud burst of the vines. This means that the chances for new infections arising from cleistotecia during the season are low and seems to contradict with the fact that we did not find identical isolates. More work is needed to understand those findings.

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Key words

Erysiphe necator, RAPD, flag shoots, cleistotecia, genetic variability

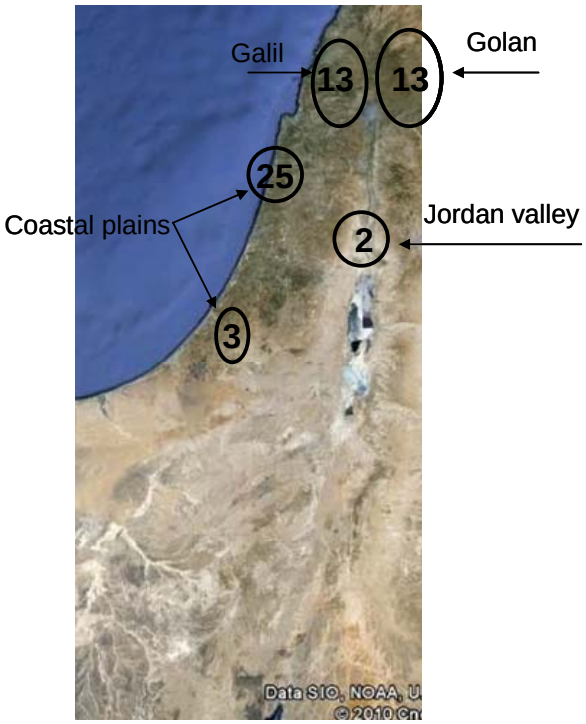


Figure1: A map of Israel showing the number of isolates collected in each region:

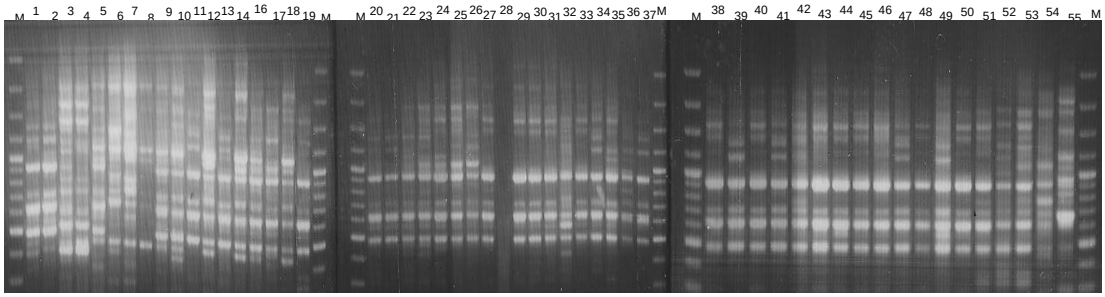
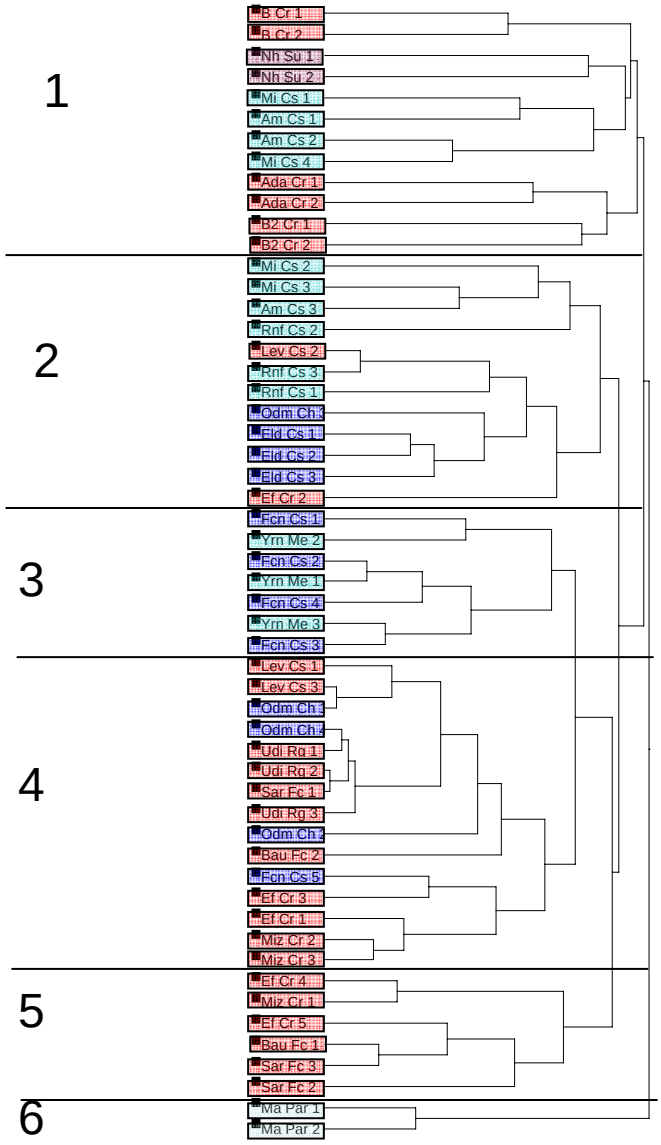


Figure 2: Amplicons generated with Operon E7 (2007 isolates).

Figure 3: Dendrogram based on 4 RAPD primers of the isolated collected in 2007.

Different colors represent the different geographical regions:
Central coastal plains, South coastal plains, Golan, Galil, Jordan valley



Multilocus genotyping of CAA fungicide resistant and susceptible grapevine downy mildew isolates infer a lack of population differentiation at both temporal and spatial scales

V. Machefer[a], S. Ahmed[a], MP. Latorse[b], R. Beffa[b], F. Delmotte[a]

[a]INRA, UMR 1065 SV, Institut des Sciences de la Vigne et du Vin, Centre Bordeaux-Aquitaine, BP 81, 33883 Villenave d'Ornon cedex, France[b] Bayer SAS, Bayer CropScience, 14 impasse Pierre Baizet, F-69263 Lyon Cedex, France

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Plasmopara viticola (Berkl. and Curt.) Berl. and de Toni., the causal agent of grapevine downy mildew disease, is a native species from North America that is believed to have been introduced into Europe in the late 1870s. The disease then spread rapidly through most of continental Europe to become one of the most important vineyard diseases today. At present, chemical control is the most effective measure used to protect grapes from downy mildew disease.

In response to the systematic control of this disease based on fungicide treatments, the pathogen has evolved genetic resistance for several classes of fungicides. Since fungicide development is a lengthy and expensive process, increasing our knowledge as to the mode and rate of evolution of resistance is of great importance. The development of effective strategies for the control of pathogen populations therefore requires precise understanding of the conditions under which genetic resistance to fungicides appear, how this trait is transmitted and subsequently maintained in natural populations.

The class of carboxylic acid amide (CAA) fungicides includes mandipropamid, benthiavali-carb, dimethomorph, flumorph, iprovalicarb and valifenalate. Resistance to CAAs in *P. viticola* field populations has been reported in France and Germany for almost 10 years, with cross resistance shown between the representatives of this fungicide family. The mode of action of CAA has been recently described by Blum *et al.* (2010) who have demonstrated that one recessive mutation (G1105S) in PvCesA3 gene (putative cellulose synthase) causes inheritable resistance to the CAA fungicide.

In this study, using neutral nuclear markers we have investigated the genetic variability of a set of samples of *P. viticola* that have been subjected to CAA treatment. Here, we compare the microsatellite diversity of groups of samples that are either resistant or susceptible to CAA. We have performed a population genetic analysis of the data in order to investigate the existence of population partitioning in resistance and susceptible individuals at both temporal and spatial scales. We also compare the genetic diversity of samples collected from different treatment trials in order to determine whether there is any difference between *P. viticola* diversity in treated and untreated areas.

Materials and methods

Sampling design. A vineyard located at Bligny in Champagne (France) was divided into three large plots of minimum 400 m^2 corresponding to three different management strategies: the first plot was the control plot (untreated), the second plot was treated with a non CAA mixture called Mikal flash (fosetyl-aluminium and Folpel) and the third plot was treated with sirbel UD, a CAA fungicide mixture (Iprovalicarbe and Folpel). These fungicides were used with a rate of application of 4 kg/ha and 1.3 kg/ha respectively. For each year, fungicides were applied three times after the first sampling date of collection, with an interval of 14 days between each treatment. Therefore, isolates of *P. viticola* collected at the second date of each year correspond to isolates that have been treated with the respective fungicide (Mikal Flash or Sirbel UD). It is worth noting that, in this protocol, the fungicides were applied in eradicant situations.

From these three plots, a total of 923 isolates of *P. viticola* were collected from single lesions on five occasions during three consecutive years (Table 1).

Fungicide sensitivity tests. Leaf disk bioassays have been used to characterize the isolates for their sensitivity to CAA. Inoculum from each single lesion was first multiply by one biological cycle onto leaves of *Vitis vinifera* cv. Cabernet Sauvignon.

A discriminatory rate of iprovalicarb (30 mg/l) was selected and mixed with the sporangia suspension of each isolate finally adjusted at 20000 sporangia per ml. Two 10 µl droplets of inoculum and fungicide were inoculated at the lower surface of each leaf disk put on survival medium (agar + 1% kinetine) in Petri dishes and placed at 20°C in climatic chamber with the photoperiod of 12 hours. Two days after inoculation the water of each droplet was suck up and five days later the growth and sporulation of the fungus were visually assessed. The isolates were classified in sensitive phenotype when no visible symptoms could be observed and resistant phenotype when efficacy was < 100%.

Table 1: Number of *Plasmopara viticola* isolates collected in Bligny vineyard according to the three plot management strategy (Non CAA treated, CAA treated, Untreated control) and to the date of the collect. Control plot is untreated, non CAA treated plot is treated with Mikal flash (fosetyl-aluminium and Folpel) and CAA treated plot is treated with sirbel UD (Iprovalicarbe and Folpel). For the CAA treated and the non-CAA treated plots, three fungicide treatments were applied between the first and the second sampling dates of collection of each year.

	07/06/2006 pre-treatment	10/07/2006 post-treatment	12/06/2007 pre-treatment	02/08/2007 post-treatment	01/07/2008 pre-treatment	Total
Non CAA treated	33	28	77	65	93	296
CAA treated	32	31	69	81	98	311
Untreated control	23	30	83	77	103	316

Multilocus genotyping. A subset of 402 isolates was selected in order to investigate the partitioning of resistant and sensitive *P. viticola* populations (Table 2). Isolates were extracted from infected plant tissue as described by Delmotte *et al.* (2006) and genotyped using eight polymorphic microsatellite markers (Delmotte *et al.,* 2006; Gobbin *et al*., 2003) and three single nucleotide polymorphism derived from an expressed sequence tag library provided by Bayer CropSciences (Table 3). Microsatellites analysis was automated on a Beckman Coulter Ceq 8000 capillary sequencer and SNPs were transformed into cleaved amplified polymorphism sequence (CAPS) markers.

Statistical analyses. The numbers of resistant and sensitive isolates and their spatial distribution in their respective plot was plotted for each date (Figure 2) and a pairwise chi squared tests were performed to compare plots at each sampling date. Departures from Hardy-Weinberg expected frequencies, observed and expected heterozygosity, allelic richness, pairwise *F*st (Weir and Cockerham, 1984) and pairwise linkage disequilibrium were measured in Genepop v4 (Raymond et Rousset, 1995). The presence of repeated multilocus genotypes was detected using Genclone 2.0 (Arnaud –Haond and Belkir, 2007).

A hierarchical analysis of molecular variance (AMOVA) was performed in ARLEQUIN version 3.11 (Schneider *et al*., 2000) in order to measure the level of genetic differentiation between fungicide resistant and susceptible individuals and also between temporal sampling efforts within the two respective groups. This was then repeated for different plots within the two main group

Table 2: Number of *Plasmopara viticola* isolates selected for the population genetic structure analysis according to their susceptibility to CAA fungicide (R= resistant to CAA, S=sensitive to CAA).

	07/06/2006		10/07/2006		12/06/2007		02/08/2007		01/07/2008		Total
	R	S	R	S	R	S	R	S	R	S	
Non CAA treated	6	23	8	18	8	15	6	3	23	1	111
CAA treated	11	21	28	2	16	23	15	8	27	10	161
Untreated control	5	13	11	16	13	18	11	11	25	7	130

Table 3: Characteristics of the three SNP (Single Nucleotid Polymorphism) that were transformed into CAPS (Cleaved Amplified Polymorphism Sequence) markers.

Locus Name	Homology	Primers sequence (5'-3')	Size (pb)	Restriction enzyme	Allele 1		Allele 2	
					Identity	Size (bp)	Identity	Size (bp)
Pvi1	Hypothetical protein	L: CCGTGACTCCCTTGTATTCC R: AACGAATAGGGTGCGTAGGA	494	P*vu* I	C	302/192	T	494
Pvi12	Ubiquitin	L: CTGACGGGCAAGACCATTAC R: GAACACACCAGCACCACACT	372	EciI	G	209/163	A	372
Pvi13	Peptidyl-prolyl isomerase	L: CCAAGTCGCAAGCAAGTAAA R: GCGAAAAAGGAAAAATAAGCA	638	HgaI	C	472/211	A	638

Results

The incidence of grapevine downy mildew infections in Bligny was relatively low in the year 2006 which enabled the exhaustive sampling of isolates, but high in 2007 and 2008. The number of resistant isolates sampled overall in the field increased over the three years of the study (Figure 1). However, this overall general trend masks the variation that exists in the number of resistant isolates found among plots for temporal sampling events. The proportion of resistant isolates collected in June 2006 (before treatment) was low and we detected no significant difference between each plot (P=0.18). Collections from July 2006 (after treatment) showed a higher proportion resistant isolates in the CAA plot compared to non CAA treated plot and untreated control plot. In June 2007 (before treatment and early in the season) samples revealed a decrease in the number of resistant isolates compared to the previous date but with no significant difference detected between plots. In August 2007, the samples collected 6 weeks after the treatments showed a higher proportion of resistance in the CAA treated plot than in the non CAA treated plot. In July 2008 (before treatment but late in the season), the mean proportion of resistant isolates overall reached 80%, with a slightly higher percentage being found in the non CAA treated plot compared to the CAA and untreated plots. For all the three years for which data were collected, we found no spatial aggregation of resistant or susceptible *P. viticola* isolates in the field.

The genotypic data were then grouped into two populations according to whether they were susceptible or resistant to CAA (called S or R respectively). Based on the genotypes derived from 11 nuclear markers, we identified 166 different multilocus genotypes among the 213 resistant isolates (G/N=0.78) and 173 among 189 sensitive isolates (G/N=0.91). Multicopy genotypes were represented no more than two or three times, except in 2008 where one resistant genotype was found in 17 other isolates. The level of genetic diversity estimated for R and S populations at each temporal sampling event showed no significant deviations from Hardy-Weinberg expectations. The number of significant tests for linkage disequilibrium detected was also low implying a largely sexual breeding system for both resistant and sensitive populations (Table 4).

Pairwise F_{ST} estimates between resistant and sensitive populations were low and not significant (excepted for July 2008) revealing little or no differentiation between the resistant and susceptible populations. Clustering analysis in the program Structure also suggests a single population.

For the hierarchical analysis, when isolates were grouped according to their susceptibility to CAA and subpopulations formed according to temporal sampling events, we found that only 0.3% of the total genetic variance could be attributed to divergence between the two main groups and 2.4% to differentiation within groups but among temporal population (Table 5). The same analysis performed with two groups (R/S) and populations represented by the treatment plots, revealed that 0.85% and 0.19% of the genetic variance resulted from group and population effect respectively, suggesting that the observed diversity can be explained at the individual level rather than between resistant or susceptible groups, or temporal and treatment plot subpopulations.

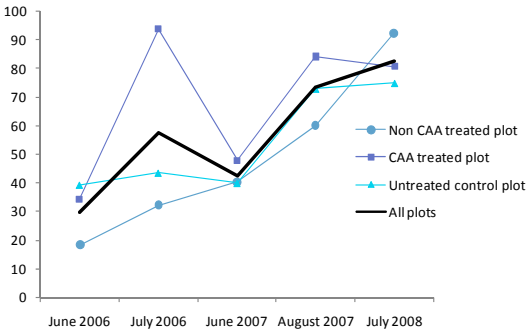

Figure 1: Time course evolution of the frequency of resistant *P. viticola* isolates within Bligny field according to the plot management strategy.

Discussion

Many interacting factors are involved in the emergence and spread of alleles that confer resistance to fungicide treatment in pathogen populations. The evolution of fungicide resistant alleles is dependent on the mode of action of the fungicide, the selection pressure on the pathogen response to the fungicide, the abiotic conditions for disease development, and the potential for evolving genetic resistance in the pathogen (such as breeding system, recombination rate, mutation rate, dispersal capability).

In this study, we have shown that the number of resistant isolates sampled increased globally over the three years in all plots (Figure 1). To allow the collect of representative number of single lesions at the beginning of the season, fungicide applications were used in eradicant situations (i.e. when disease epidemics is already in its exponential growth), a situation that did not fit classical agronomical practises. This result reinforces the recommendation to avoid applying CAA fungicides after downy mildew infections have started, especially in situations where resistance to the fungicide is suspected. It is also possible that, in 2008, a year that presented favourable conditions to the initiation of downy mildew epidemics, dispersion from surrounding vineyards where CAA fungicides have been intensively used may have contributed to increase the frequency of R isolates in the Bligny vineyard.

When one considers each temporal group in greater detail, the number of resistant isolates sampled was significantly higher in the CAA plot only after the treatment in 2006 (Year 1). Conversely, in 2008 (Year 3) before treatment in the target plot but after treatment in the surrounding vineyards, we found that the number of resistant isolates was greater in the non CAA treated plot compared with the other two. In terms of spatial genetic structure we found that CAA treatment does not affect the spatial distribution of resistant isolates (Figure 1) and no spatial aggregation was observed. These results are congruent with dispersal of *P. viticola* at the meters scale leading to population mixing across the plots.

The genetic diversity we observe in *P. viticola* could almost entirely be attributed to individual differences rather than temporal or plot differences being they resistant or susceptible to CAA fungicide.

Table 4: Comparison of genetic characteristics of resistant vs. sensitive populations of *P. viticola*.

	R						S					
	07/06/2006	10/07/2006	12/06/2007	02/08/2007	01/07/2008	Total	07/06/2006	10/07/2006	12/06/2007	02/08/2007	01/07/2008	Total
N	22	47	37	32	75	213	57	36	56	22	18	189
MLG	19	42	36	32	47	166	55	36	53	21	17	173
G/N	0.86	0.89	0.97	1	0.63	0.78	0.96	1	0.94	0.95	0.94	0.91
S	17	38	35	32	40	145	53	36	50	20	16	14
Max	3	3	2	-	17	17	2	-	2	2	2	4
Allelic richness	2.09	2.36	2.36	2.45	2.27	2.54	2.36	2.45	2.64	2.18	2.36	2.90
He	0.28	0.31	0.32	0.33	0.33	0.33	0.31	0.32	0.33	0.34	0.31	0.33
Ho	0.37	0.36	0.35	0.34	0.40	0.37	0.38	0.37	0.34	0.35	0.33	0.36
$P_{deficit}$	0.99	0.97	0.94	0.3	1	1	0.83	0.7	0.01	0.6	0.29	0
P_{excess}	0.02	0.03	0.06	0.7	0	0	0.17	0.32	0.99	0.41	0.7	1
LD	0/55	3/55	2/55	1/55	23/55	23/55	2/55	2/55	5/55	2/55	3/55	4/55

N: number of individual, MLG: multilocus genotype, G/N: number of multilocus genotype/total number of individuals, S: number of singleton, Max: maximum number of repetition on a MLG, Allelic richness: average number of allele, He/Ho : expected and observed heterozygositie, *P* : excess and deficit heterozygositie, LD : linkage disequilibrium.

Table 5: Hierarchical analysis of molecular variance for samples of *Plasmopara viticola* grouped by their susceptibility or resistance to CAA (groups) and by date or by plots (populations).

Source of variation	Degrees of freedom	Variance components	Percent of variation	Fixation indices
Among groups (R/S)	1	0.006	0.34	F_{CT}=0.00336 *
Among dates within groups	8	0.045	2.48	F_{SC}=0.02486 *
Within populations	794	1.781	97.2	F_{ST}=0.02813 NS
Among groups (R/S)	1	8.555	0.85	F_{CT}=0.00854 NS
Among plots within groups	4	0.003	0.19	F_{SC}=0.00188 *
Within populations	798	1.813	99,0	F_{ST}=0.01040 NS

F_{CT}: estimated fixation index among differnet groups; F_{SC}: estimated fixation index within a region; $F_{ST:}$ estimated global fixation index within populations

* P<0.01, NS: not significant

With regard to breeding system, we know that this pathogen has the capacity for both sexual and asexual reproduction. Analysis of the genotype data revealed no significant deviations from Hardy Weinberg expected frequencies and linkage disequilibrium was low. In addition, we found a low number of multiple matching multilocus genotypes, inferring a low incidence or absence of clonal reproduction except at year 3 after treatment (2008) (Table 4). Together these observations would suggest that the breeding system in ours plots is random and predominantly sexual between resistant and susceptible isolates. These results also confirm the importance of primary inoculum in the epidemics of grapevine downy mildew.

In conclusion, this study suggests a lack of both spatial and temporal genetic structure between R and S populations of *P. viticola*. No genetic structure was detected in the set of genotyped samples, suggesting a single panmictic population where all isolates have the potential to breed with each other regardless of fungicide susceptibility and their plot origin. Therefore, in the absence of fungicide selection pressure, the wild type (S) and the mutated (R) alleles are fully reshuffled into *P. viticola* genotypes by the sexual reproduction. Since the resistance to CAA has been described as recessive (Blum *et al*. 2010), an annual generation of sexual reproduction results in a decrease of resistant isolates (RR) due to the formation of heterozygotes genotypes (RS) that are sensitive. Therefore, rotation with other fungicide compounds having other modes of action is providing a good strategy to manage resistance to this class of fungicide.

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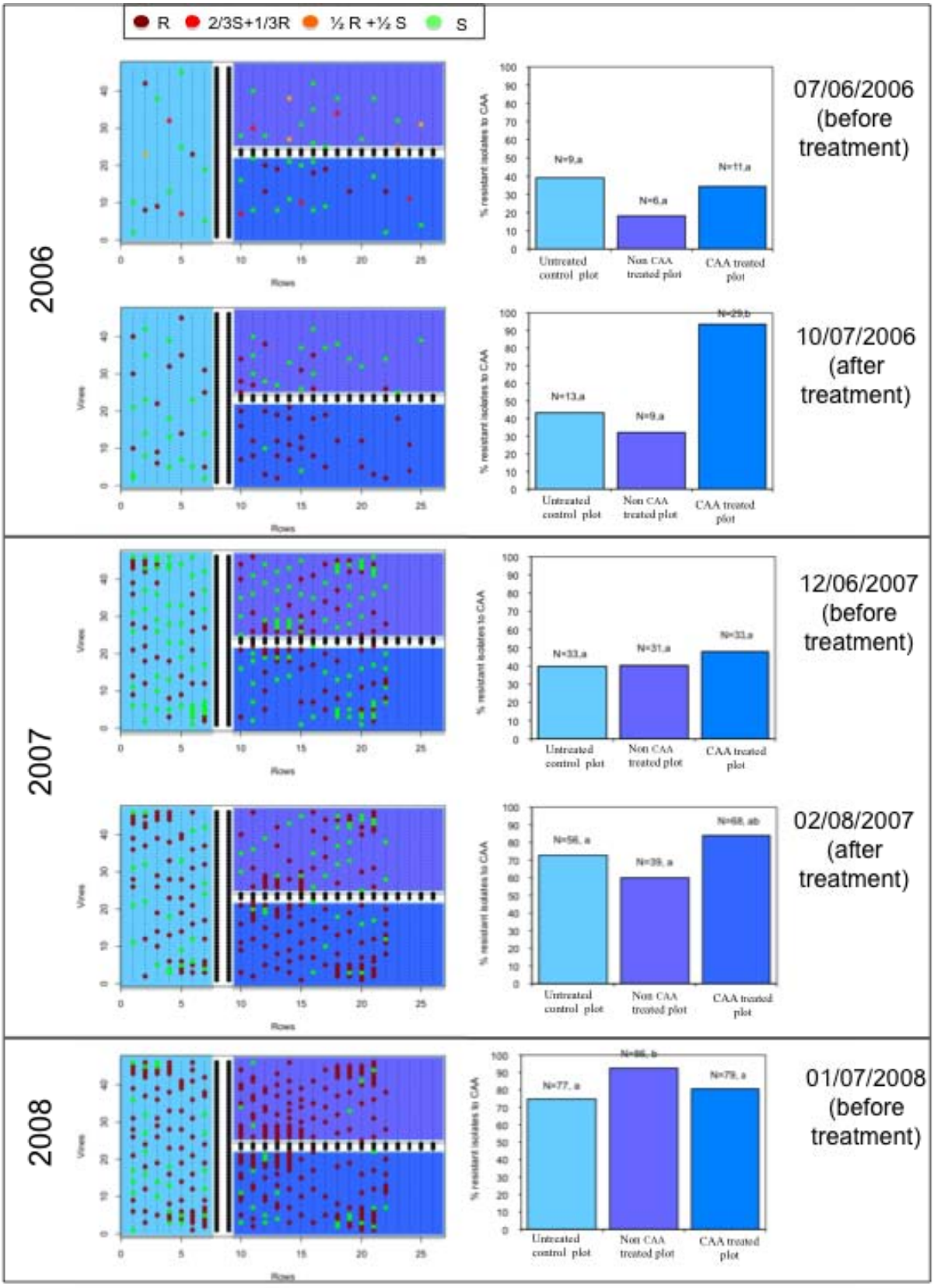

Figure 2: Spatial representation of the *P. viticola* isolates collected within the vineyard at the five dates (green=sensitive; red=resistant). The graph represents the percentage of resistant to the CAA fungicide for each date, depending on the 3 treatment modalities. The date 01/07/2008 is before treatment on the target plot (but after treatment in the surrounding vineyards).

Session 3: Detection methods, monitoring, epidemiological modelling, disease management

Aerobiology of *Erysiphe necator* in northern viticulture

O. Carisse[a], A. Lefebvre[a], M. Tremblay[a]

[a] Agriculture and Agri-Food Canada, Horticultural Research and Development Centre, 430 Gouin Blvd., Saint-Jean-sur-Richelieu, Québec, Canada, J3B 3E6

The production of grapes and wine in Quebec is a key component of agrotourism. Most of these cultivars are susceptible to powdery mildew, caused by *Erysiphe necator* (Schw.), the key disease for scheduling fungicide applications in Quebec.

Much of what is know on the powdery mildew epidemiology, yield losses and management tactics was derived from studies of powdery mildew on European cultivars or in areas with warmer winter climates than in the province of Quebec (Gadoury *et al.*, 2001, Jailloux *et al.*, 1999; Willocquet and Clerjeau, 1998; Willocquet *et al.*, 1998). In Quebec, growers grow either winter hardy cultivars such as Frontenac, (*Vitis Riparia* x Landot 4511) or hybrids such as Chancellor or Geisenheim that are covered with soil during the winter months. In absence of knowledge of powdery mildew epidemic on these cultivars and under northern conditions, management is largely based on routine use of fungicides. Field observations revealed that, under conditions in Quebec, the time of powdery mildew onset can vary from mid-June to early August. Hence a fungicide spray program initiated soon after budbreak may result in unnecessarily fungicide sprays. These observations stimulated the idea that a degree-day model coupled with airborne inoculum monitoring might be used to time fungicide spray.

Tactical crop protection decisions based on assessments of disease intensity or incidence is a key element of integrated pest management. Basing pest management decisions on current or predicted disease level or on the size of the pathogen population is, in many cases, a sound approach. Such an approach has been used to reduce pesticide use, which, in turn, generally reduces pest control costs, negative impacts on the environment and is a key component of fungicide resistance management strategy (Campbell and Madden, 1990). For grape powdery mildew, making decisions based on disease monitoring could be cumbersome mainly at low disease levels. At the early stage of epidemic development, lesions are not numerous and sometimes difficult to detect and distinguish from pesticide residues.

This study is part of a larger program aiming at acquiring knowledge on epidemiology of grape powdery mildew under the conditions of Quebec and at developing management tools adapted to our area and cultural practices. Therefore, the objectives of this study were two-fold: 1) develop and validate a model to estimate proportion of seasonal inoculum in relation to degree-days; and 2) model the relationship between airborne conidium concentration and powdery mildew incidence progress and test an action threshold based on airborne inoculum concentration to time intervals between fungicide applications.

Degree-day model parametrization and validation

Data were collected with a Burkard sampler in 2000 to 2002 and with impaction samplers in 2000 and 2001, for a total of 5 data sets (season-years) used for the model identification of parameters. For each year and cultivars, the airborne inoculum was expressed as the proportion of the total spores captured over the entire season (Pmaxacc). To validate the model, additional data were collected in 2003 and 2004. The experiment was conducted in vineyards planted with the cultivars Chancellor, Frontenac and Geisenheim. Cumulative degree-days (Base 6oC) were calculated by summing daily degree-days from the vine growth stage 7 (2 or 3 expanded leaves on Eichhorn-Lorenz scale). To describe the proportion of cumulative airborne conidia concentration (Pmaxacc) as a function of degree-days, various non-linear sigmoid models were tested (Carisse *et al.*, 2009c) among which the Richards model was expressed as:

$$\text{Pmaxacc} = K * (e^{-r * DD})^{(1/1-m)}$$

where, *DD* is degree-days in base 6oC accumulated from growth stage 7, *K* is the upper asymptote, *r* is the rate of increase in Pmaxacc with increasing DD, and *m* is a shape parameter.

The degree-day model (later called DD model) was developed based on the premise that only the exponential phase of the disease significantly affects yield losses and hence fungicides are not needed before the onset of this phase. Therefore, action thresholds were arbitrarily set at a proportion of cumulative airborne conidia concentration (Pmaxacc) of 0.01 and 0.05 for highly and moderately susceptible cultivars, respectively. The field evaluation experiment was conducted with three grape cultivars, Chancellor in 2004 to 2007, and Frontenac and Geisenheim from 2005 to 2007. Each year and for all cultivars, the experimental plot was 36 x 36 meters divided into four sections of 9 m x 36 m (3 rows of 48 vines). In each of the sections, one of the following fungicide spray schedule was applied, 1) no fungicide which served as a control; 2) fungicide applied at 7 to 10 day interval starting at the 3-4 leaves growth stage; 3) a fungicide spray program (as in treatment 2) initiated when the model predicted that 1% (Chancellor and Geisenheim) or 5% (Frontenac) of the seasonal cumulative airborne conidia concentration (Pmaxacc) was reached; 4) a fungicide spray program (as in treatment 2) initiated when predicted Pmaxacc reached either 1% (Geisenheim) or 5% (Chancellor and Frontenac) and daily spore catch reached 50 conidia/m3air/h. For treatment 3 and 4, once the fungicide spray program was initiated the other fungicides were applied according to the calendar schedule treatment 2.

Relationship between airborne conidium concentration and powdery mildew incidence

The experiment was conducted from 2004 to 2007 in a vineyard planted with three grape cultivars, Chancellor, Frontenac and Geisenheim. Airborne conidium concentration was monitored three times per week using rotating-arm impaction spore samplers placed at 45 cm above the ground. To describe the pattern of powdery mildew incidence (PMI) as a function of cumulative airborne conidia concentration (CACC), selected response models were fitted to the data (Carisse *et al.*, 2009b). Among which the cumulative form of the Weibull function expressed as:

$$PMI = a + b(1 - \exp(-(CACC + d(\ln 2)^{(1/e-c)})^e))$$

d>0, e>0, $CACC > c - d * (\ln 2)^{1/e}$

where *a* is the intercept, *b* is the upper limit of the response (upper asymptote), *c* is the transition centre and *d* and *e* are regression parameters used to calculate the transition width as:

$$2^{(1/e)} * d\,(\ln\ 2)^{(1/e)} - d\,(2\ \ln\ 2 - 3\ \ln\ 3)^{(1/e)}$$

For each cultivar, the parameter identification for the ACC model was based on data collected in 2004-2005-2006, and validation of the model was based on independent data collected in 2007.

Results

The field evaluation experiment was conducted in 2007 and 2008 in a vineyard planted with the cultivar Chancellor. Four fungicide spray schedules were compared: 1) no fungicides applied which served as a control; 2) fungicides applied at 7 to 10 days intervals starting at the three to four leaf growth stage (standard); 3) a fungicide spray program (as in treatment 2) initiated when 1% of the seasonal cumulative airborne conidia concentration (Pmaxacc) was predicted based on the degree day model or at 25% bloom whichever arrived first; 4) a fungicide spray program initiated as in treatment 3 but with interval between sprays based on airborne inoculum concentration at a threshold of daily spore catch of 50 conidia m^{-3} of air. Each treatment was replicated four times for a total of 16 plots arranged as a randomized complete block design.

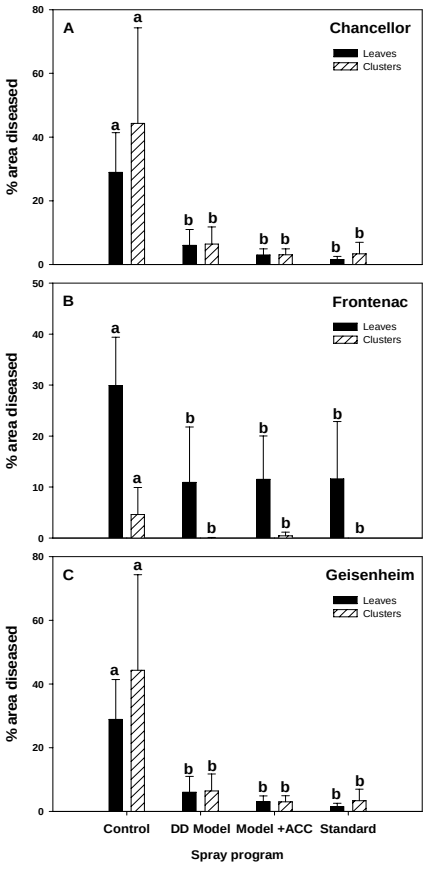

Figure 1: Mean grape powdery mildew severity on leaves (black) and on clusters (striped) of cultivars Chancellor, Frontenac and Geisenheim monitored in unsprayed plots (control) and in plots sprayed according to a standard program, to the degree-day model and to both the degree-day model and airborne conidia concentration (Model+ACC).

Depending on years and cultivars, the use of the model reduced the number of fungicides sprays by 40 to 55%. The input data are hourly temperatures, from which the degree-days are calculated and the proportion of seasonal inoculum estimated. The degree day model could be used as a component of a risk management system for grape powdery mildew to estimate the need for fungicide sprays before bloom or to time the initiation of a fungicide spray program.

The relationship between incidence of powdery mildew on the leaves of three cultivars (Chancellor, Geisenheim, and Frontenac) and the cumulative concentration of airborne conidia (based on 3 days of sampling weekly) was then studied. This relationship was similar for the three cultivars (R^2=0.97, 0.95, 0.97, for cvs Chancellor, Geisenheim, and Frontenac respectively) and was well described using the cumulative form of the Weibull model (Figure 2). Based on this model, it was possible to establish the period of high risk (highest increase in powdery mildew incidence) from 645 to 5614, 2437 to 2951, and 1052 to 3061 conidia m^{-3} of air for the cvs Chancellor, Geisenheim, and Frontenac, respectively.

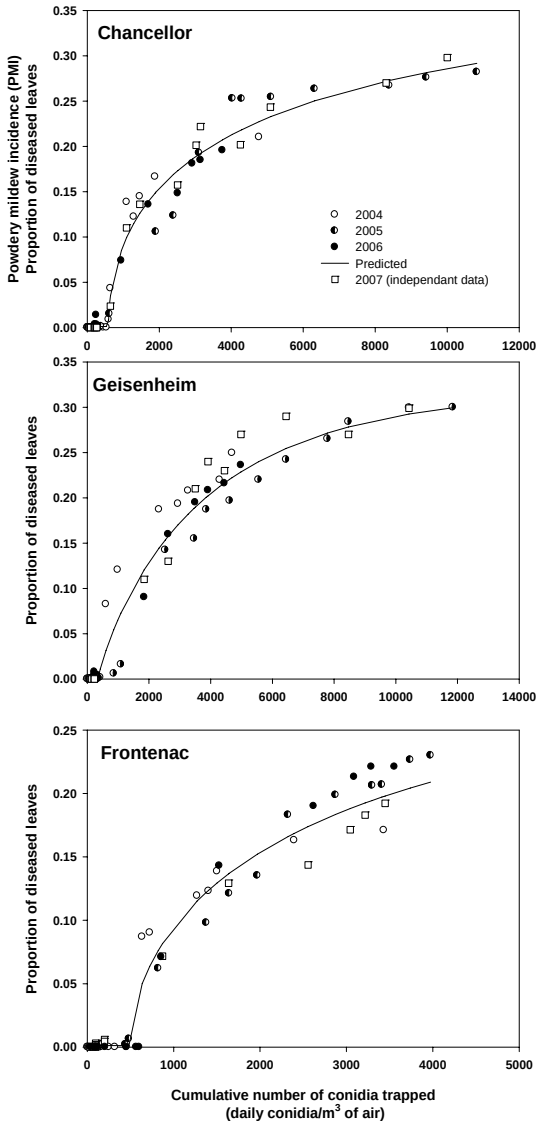

Figure 2: Mean powdery mildew incidence (proportion of diseased leaves) in relation to cumulative number of conidia trapped in a vineyard of the cvs Chancellor, Geisenheim and Frontenac. The symbols are field

observations and solid lines are from values calculated from the models.

An action threshold, for timing interval between fungicide sprays, of 50 conidia m^{-3} day^{-1} was evaluated under field conditions with cv Chancellor and was as good as a calendar-based program with fewer fungicide sprays under unfavourable weather conditions. Monitoring airborne inoculum could be used as a component of a risk management system for grape powdery mildew to time intervals between fungicide sprays.

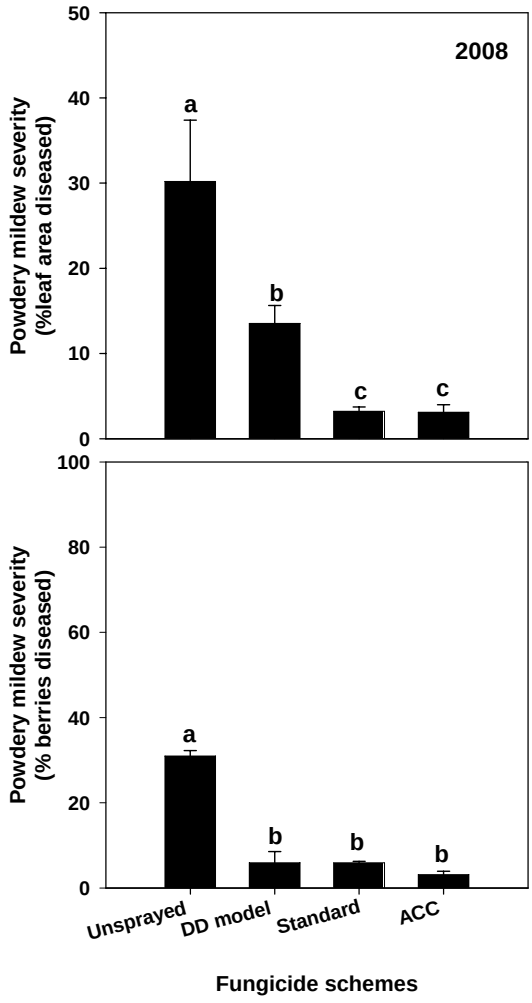

Figure 3: Mean grape powdery mildew severity on leaves and on clusters of cv Chancellor observed in 2008 unsprayed plots and in plots sprayed at 7 to 10 d intervals starting at the 3-4 leaf growth stage, in plots sprayed at 7 to 10 d intervals starting when 1% of the seasonal cumulative airborne conidia concentration (Pmaxacc) was predicted at 25% bloom whichever arrived first, and in plots sprayed at intervals between sprays based on airborne inoculum concentration at a threshold of daily spore catch of 50 conidia m-3 of air initiated as in treatment 3.

The approach investigated in this study is based on the premise that it is possible to accurately and economically monitor airborne spores. There are several constraints associated with using spore traps among which is the cost of the sampler itself, cost of labour to collect the rods or tapes (spore collecting support) and quantification of spores. These samplers are already used for routine monitoring of airborne spores of *Botrytis squamosa* (Carisse et al, 2009a), and as a tool for optimizing the timing of fungicide sprays. In addition, Falacy *et al.* (2007) recently developed an essay based on polymerase chain reaction to detect and quantify airborne spores of *E. necator* from a vineyard sample collected using rotating-arms samplers making this technology accessible for practical disease management. In the near future, it is possible to envision the deployment of powdery mildew disease prediction models combined with in field inoculum monitoring. Advances in information and fungal detection technologies are making it possible to design plant disease prediction systems that integrate near real-time environmental and inoculum data and information on the status of the crop and disease within individual fields provided by local monitoring networks.

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A multiplex polymerase chain reaction assay for the detection and identification of *Plasmopara viticola*, *Erysiphe necator* and *Botrytis cinerea* spores in airborne environmental samples

V. Huerga, A.M. Díez-Navajas

NEIKER-Tecnalia. Department of Plant Production and Protection. Box 46. E-01080 Vitoria-Gasteiz, Spain, e-mail address: vhuerga@neiker.net, adiez@neiker.net

Introduction

Three important pathogens affecting grapes are the two specific obligate biotrophic agents *Erysiphe necator* [*Uncinula necator* (Schw.) Burr.] and *Plasmopara viticola* (Berck. & M. A. Curtis) Berl. & De Toni in Sacc., causing powdery and downy mildew, respectively, and the necrotrophic pathogen *Botrytis cinerea* Pers., which causes grey mold. All decrease yield and fruit quality and lead to important economic losses. Air is probably the most important agent for the transport of spores of these pathogenic fungi. Rapid tools for monitoring the presence and the severity of the infection in the vineyard, before the symptoms appear, would help growers to apply chemicals more sparingly, without the risk of crop loss (Cséfalvay *et al.*, 2009). Passive traps, consisting on microscopic slides coated with an inertial sticky substance (Iturritxa and Ganley, 2007) are the simplest method to collect airborne biological particles. These traps are observed under microscope to identify the spores of interest.

The Polymerase Chain Reaction (PCR) has become an accepted technique for the identification of airborne fungal spores (Williams *et al.*, 2001; Chen *et al.*, 2002; Haugland *et al.*, 2002; Dean *et al.*, 2004, 2005; Kerwani *et al.*, 2005; Karakousis *et al.*, 2006) as an alternative to the traditional methods relying on microscopic observations, which can be time-consuming and laborious. PCR provides a rapid and sensitive method for fungal identification. The Multiplex Polymerase Chain Reaction (PCR) allows the simultaneous identification of two or more target sequences by including more than one pair of primers in the same reaction. This technique saves time and effort in the laboratory (Henegariu *et al.*, 1997; Markoulatos *et al.*, 2002).

The present study describes a rapid PCR assay that allows the simultaneous detection and identification of *E. necator, P. viticola* and *B. cinerea* spores collected from a single airborne environmental sample.

Materials and methods

Fungal isolates and harvest of spores: spores of *P. viticola* and *E. necator* were harvested directly from fresh plant leaves by soaking these in sterile distilled water. *B. cinerea* was collected from infected grapes and grown in PDA Petri dishes, maintained in a growth chamber during 14 days at 21 ºC with a photoperiod of 16/8 hours (day/night). Spores were harvested from PDA in the same way as for *P. viticola* and *E. necator* from vegetal tissue. The number of spores was measured under a visible microscope with a hemocitometer and dilutions made with distilled water if necessary. They were preserved at -20 ºC for later assays.

Sample preparation and DNA extraction: a known number of each fungal spore was laid on petroleum jelly-coated slides. Once the drops were air-dried, the petroleum jelly was collected with a sterile stick in a micro-tube. DNA extraction was performed just after sample preparation as described by Chen (2002) with some modifications. Briefly, 15 µl of sterile distilled water was added into the micro-tube containing the sample and the same volume of beads (Glass beds, acid-washed < 106 µm. SIGMA). The mixture was homogenized at 30 sg^{-1} for 3 min, heated in a boiling water bath for 10 minutes, then cooled on ice during 10 minutes and finally spun at 14,000 rpm for 30 seconds at room temperature. The petroleum jelly was eliminated and the supernatant was directly used as template for PCR.

Primers selection: To identify *E. necator*, primer pair Uncin144 (5´-CCGCCAGAGACCTCATCCAA-3´) and Uncin511 (5´-TGGCTGATCACGAGCGTCAC-3´) (Fallacy *et al.*, 2007) was used, which generated a 367 bp fragment. For *P. viticola* identification, we used the primers GiopR (5´-TCCTGCAATTCGCATTACGT-3´) and GiopF (5´-GGTTGCAGCTAATGGATTCCTA-3´) (Valsesia *et al.*, 2005). These generated a 208 bp long PCR product. For the detection of *B. cinerea*, the primer pair BOTYF4 (5´-CAGCTGCAGTATACTGGGGGA-3´) and BOTYR4 (5´-GGTGCTCAAAGTGTTACGGGA-3´) was selected, which generated a 533 bp fragment (Ma & Michailides, 2005). After the multiplex PCR reaction, a pair of internal primers of the sequence generated by the BOTY primer pair was designed with the program Primer3 (http://www.frodo.wi.mit.edu/primer3) in order to perform a nested-PCR. So, the internal primers were BOTYF´ (5´-GATCCATCACTCCCACCACT-3´) and BOTYR´ (5´-CCTCTAGGGTCACGTGGAAG-3´), generating a 235 bp fragment.

PCR conditions: before multiplex PCR analysis, each organism was subjected to individual PCR to optimize the conditions necessary for sufficient amplification and to verify the specificity of each primer pair. The emphasis was on generating a positive result for the three organisms using the same constituents and PCR conditions so as to make a simultaneous detection possible.

Each reaction mixture (25 µl) contained 10 µl of the target DNA, 2.5 µl 10x PCR buffer, 3 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM of each primer pair, 0.5 µg/µl of BSA and 1.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR reactions were performed with one cycle at 94 ºC for 5 min, followed by 35 cycles at 94 ºC for 40 sec, 60 ºC for 40 sec, 72 ºC for 1 min and a final elongation step at 72 ºC for 10 min. PCR products were examined by electrophoresis on 1.5% agarose gels in 1x TAE buffer.

For the multiplex PCR reaction, the buffer concentration was lowed to 0.5x, the AmpliTaq polymerase increased to 2 U, the concentration of primers were changed (Giop primer pair for *P. viticola* was lowed to 0.1 µM and the other two pairs increased to 0.5 µM) and the concentration of BSA was lowed to 0.1 µg/µl. This reaction was performed

as follows: 95ºC for 5 minutes, followed by 35 cycles of denaturation at 94ºC for 50 sec, annealing at 56 ºC for 50 sec, extension at 72ºC for 1 min and a final extension at 72ºC for 10 min. The second round of amplification to detect *B. cinerea* was carried out with 5 µl of the multiplex reaction mixture as template and the rest of components and conditions were the same as in the individual assays, apart from BSA, which was not added to the reaction mixture.

Results and discussion

PCR optimization

Individual fungal PCR: the bead-beating method resulted to be efficient in the release of DNA from fungal spores and the specificity of the specific primer pairs was corroborated, as they did not amplify any target DNA from the other organisms different from that for which they were designed (Figure 1). The addition of 0.5 µg/µl bovine serum albumin (BSA) allowed the amplification, increasing the sensitivity of *B. cinerea* and *E. necator* loci, whose sequences resulted difficult to amplify in each reaction, being their reproducibility very low. It also allowed all the reactions to be carried out in the same conditions.

Multiplex and nested PCR: two bands, corresponding to *P. viticola* and *E. necator* target genes, were detected in all multiplex reactions at the conditions described previously (Figure2). However, in all cases the band corresponding to *B. cinerea* Boty sequence was undetectable in agarose gels. In the second round of amplification, it was achieved the visualization of a band of 235 bp, corresponding to the internal fragment of Boty sequence (Fig 2, lanes 4 and 5).

Conclusions

The bead-beating method for the breakage and release of fungal DNA was essential for subsequent amplification, being less time-consuming for the extraction of nucleic acids than the conventional phenol chloroform protocol or than a commercial extraction kit.

The addition of BSA in the PCR mixture increased considerably the sensitivity in the detection of *E. necator* and *B. cinerea*.

A multiplex PCR has been established in our laboratory, as a rapid assay to detect airborne fungal spores of *P. viticola*, *E. necator* and *B. cinerea* in the vineyard, followed by nested PCR assay in the later case. It is a fast method for the simultaneous detection of these three pathogens by the PCR technology and from airborne fungal spores collected with sticky traps, to avoid microscopical observations of them, which is more time consuming and slower than molecular techniques.

Nested PCR following the multiplex reaction proved to be more sensitive in the detection of *B. cinerea* Boty locus, which turned to be difficult to amplify.

Future work would include de development of a quantitative multiplex reaction that would enable not only the identification of the three vineyard pathogens, but also to determine the amount of each fungal spore in the environmental sample.

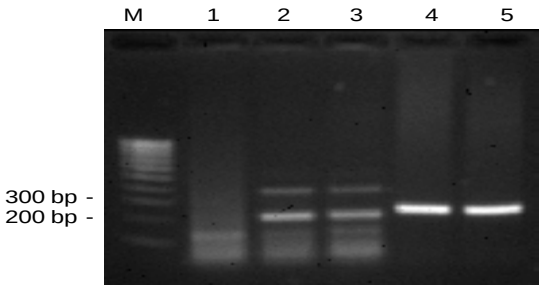

Figure 2: Multiplex and nested PCR. Lane M: DNA ladder (Hipper Lader IV, Bioline); Lane 1: water; Lane 2, 3: spores of *B. cinerea*, *P. viticola* and *E. necator* (the band of 367 bp corresponds to *E. necator* target gene, while the band of 208 bp, to *P. viticola*): Lane 4, 5: nested PCR (the band corresponds to a 325 bp fragment belonging to an internal sequence of Boty element).

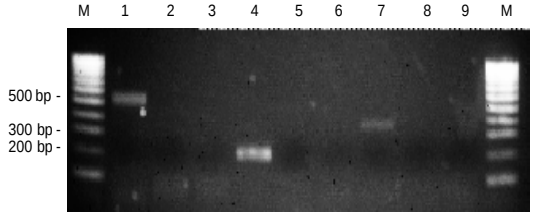

Figure 1: Results of PCR assay to test the specificity of the primers of study for *B. cinerea*, *P. viticola* and *E. necator* detection. Lanes M: DNA ladder (Hipper Lader IV, Bioline); Lane 1: *B. cinerea* with primers BOTY; Lane 2: *B. cinerea* with primers Giop; Lane 3: *B. cinerea* with primers Uncin; Lane 4: *P. viticola* with primers Giop; Lane 5: *P. viticola* with primers BOTY; Lane 6: *P. viticola* with primers Uncin; Lane 7: *E. necator* with primers Uncin; Lane 8: *E. necator* with primers Giop; Lane 9: *E. necator* with primers BOTY.

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Classical conditioning of domestic honeybees to olfactory stimuli associated with grapevine powdery mildew infections

AM. Sutherland[a], W. D. Gubler[a], R. M. Wingo[b], K. J. McCabe[b]

[a]Department of Plant Pathology; University of California, Davis; One Shields Avenue; Davis, CA 95616; USA
[b]Chemical Diagnostics & Engineering, Los Alamos National Laboratory; Los Alamos, NM 87545; USA

Introduction

Organisms with superior sensory abilities can be developed into biological sensors by utilizing innate (or learned) unambiguous responses or processes as indicators of the presence of specific compounds or (phenological) situations of human interest. This is generally accomplished through classical conditioning (aka Pavlovian conditioning: Pavlov, 1927), where an unconditioned stimulus (US), such as food, that elicits an unconditioned response (UR), is spatiotemporally coupled with a novel conditioned stimulus (CS). In time, the organism exhibits the UR when presented with the CS, in anticipation of the US. In this way, the UR becomes a conditioned response (CR), indicative of the presence of the CS. Domestic honeybees, *Apis mellifera* L., have olfactory senses comparable to those of dogs, capable of detecting specific volatile organic compounds (VOC) within a carrier gas in the low parts per billion (Wingo, Los Alamos National Laboratory, unpublished data). The proboscis extension reflex (PER) is an unambiguous innate response in honeybees to antennal contact with sucrose. Through the aforementioned technique of conditioning, bees have often been trained to associate novel VOC with a sucrose reward (Bitterman et al, 1983), exhibiting PER when the VOC comes in contact with chemoreceptors on the antennae. Attempts at commercial applications of this phenomenon have so far concentrated on detection of explosive and narcotic VOC for security purposes, at international ports-of-entry (Wingo, Los Alamos National Laboratory, unpublished data). Our objective, however, is to develop conditioned PER in honeybees into an agricultural decision support device, specifically serving to identify agricultural plant pathogens at low densities and / or seasonally early in valuable commodity systems in order to reduce economic and environmental costs due to unnecessary fungicide applications. Ideally, this would involve *in situ* detection. Grapevine powdery mildew (PM), *Erysiphe necator* Schwein [= *Uncinula necator* (Schwein) Burrill], serves as an ideal model pathosystem within which such a biological sensor could prove valuable. In California, the disease is widespread, difficult to initially detect, and managed via intensive and sometimes ineffective fungicide applications (Gubler and Hirschfelt, 1991).

The agroecosystem is a complex biological arena, far from the sterile environment of a laboratory or the controlled environment of an international airport. Accordingly, the conditioning of biological sensors for use in this system must be modified from that employed for sensors of explosives. A strong and ubiquitous background of VOC is present in an agricultural arena that is sometimes similar to the target VOC (Sutherland and Gubler, unpublished data).

Therefore, if honeybees were trained to respond to pathogen-infected leaves, it is possible that they would also respond similarly to uninfected leaves, thereby exhibiting a false positive response and essentially rendering the biological sensor useless. Whereas conditioning for detection of explosives typically involves presentation of the target VOC in a carrier gas of ambient (laboratory) air, conditioning for detection of pathogen-infected plants must utilize a background carrier gas that mimics conditions found in the intended agricultural venue. We have designed a stimulus delivery system that incorporates a constant background carrier gas consisting of the headspace captured from uninfected leaves (Sutherland et al, patent pending). We hypothesized that honeybees conditioned to PM-infected material using conventional methods would exhibit high levels of false positive responses to uninfected conspecific material, and that incidence of this problem could be reduced by employing our new conditioning methods.

Materials and Methods

Subjects. Bees were collected from hives maintained by the Bee Biology division of the Entomology Department at the University of California, Davis. Approximately 100 foraging worker bees were collected in several acrylic tubes (25-50 bees per tube) using a modified handheld vacuum (Black and Decker Inc. CHV 9608 Towson, Minnesota) at the entrance of the hive, and then immediately taken back to the laboratory and released into acetal / acrylic cages. Bees were allowed to feed (40% sugar, 60% honey) ad libitum for eight hours, after which the food was removed, mandating a starvation period of approximately twelve hours. The following day, each bee was individually restrained in a polycarbonate cylindrical harness such that only the proboscis and antennae were able to move freely. Fitness of each restrained bee was initially assessed via testing for UR ability following antennal presentation of 40% sucrose. Of those subjects exhibiting the UR, 30 each (60 total) were randomly assigned to receive either conventional or modified conditioning to PM-infected material, and were subsequently used in acquisition and discrimination trials.

Stimuli materials. The US, or reward, was antennal presentation of 40% sucrose, in all cases. The CS, or target, was the dynamic headspace of PM-infected, detached grape, *Vitis vinifera* L. 'Carignane', leaves, petioles sealed with polytetrafluoroethylene (PTFE) tape, in all cases. Pathogen was maintained in controlled environment culture on containerized three-year-old vines. Cohort vines were kept separately in greenhouse culture and maintained as uninfected via constant temperature (30^{o}C) and regular applications of volatilized elemental sulfur.

Stimulus delivery. A stimulus controller (TYPE CS-55, Syntech Research and Equipment, Kirchzarten, Germany) was used to deliver a regular pulse of the target, within a continuous flow of a carrier gas, utilizing

pulse compensation such that outlet flow was constant. Airflow was delivered through two arms of PTFE tubing, each including a glass sample tube, joined at the outlet with a borosilicate glass y-tube. Sample tubes were maintained at constant 30.0°C through use of circulating water baths, a temperature controller (JKEM Scientific, USA) coupled with a thermal blanket, and a cylindrical copper thermal ballast.

Conventional acquisition trials. One infected leaf, the target, was placed within the sample tube of the pulse delivery arm of the stimulus controller. The sample tube of the continuous flow arm was left empty. Therefore the target was presented within a carrier of ambient air only. Each restrained subject, in series, was placed on a copper stage ~3cm from the stimulus delivery system outlet (Figure 1) and exposed to a 6s presentation of the target. The reward was administered to the subject during the final 1-2s of the target pulse. Six of these trials were conducted, in rotation and series, for each subject, such that no subject encountered the target twice until all subjects had done so once.

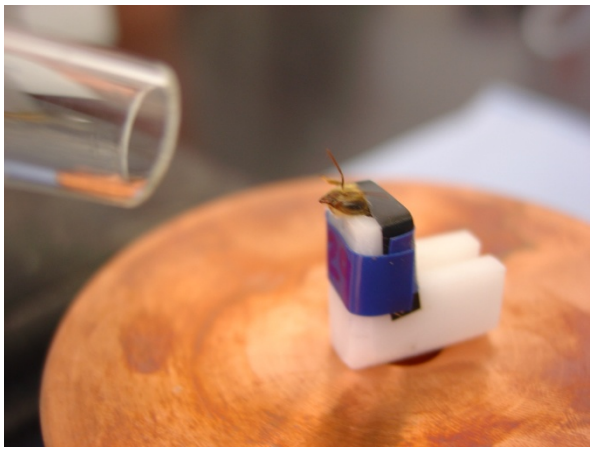

Figure 1: Restrained honeybee at the outlet of a stimulus delivery system prior to presentation of olfactory stimuli resulting from a powdery mildew infection.

Modified acquisition trials. Methods were as described above for conventional acquisition except that the sample tube of the continuous flow arm of the stimulus controller contained an excised uninfected grape leaf, petiole sealed with PTFE tape. In this way, target VOC were delivered in a carrier gas containing headspace from an uninfected leaf.

Discrimination trials. Pulse delivery arm of stimulus controller was removed and replaced with similar configuration of tubing containing an excised, uninfected grape leaf. Subjects that previously had responded to the target CS during acquisition trials were exposed three times to a 6s pulse of headspace from the uninfected leaf within a carrier gas containing the same. No sucrose reward was presented after these tests for false positive CR. Afterwards, the pulse arm was changed back to that containing the infected leaf, and subjects were exposed three times to the target followed by reward, as in acquisition trials.

Variables. The response variables in all cases were; 1) exhibition of PER in response to pulse presentation, or CR, and 2) exhibition of PER in response to sucrose presentation, or UR. These data were binary, with values of zero (0) for no response and one (1) for response. The CR could be true positive, as in acquisition trials and discrimination trials where the pulse arm contained an infected leaf, or false positive, as in discrimination trials where the pulse arm contained an uninfected leaf. Subjects failing to exhibit PER in response to the US (0 or negative UR) were deemed unfit and were removed from the experiment. Data were described as proportional means of subject responses.

Results and discussion

Successful conditioning was achieved rapidly and to a high degree in honeybees subjected to conventional acquisition trials, with 75.9% and 89.7% exhibiting positive CR during the fourth and sixth trials, respectively (Figure 2).

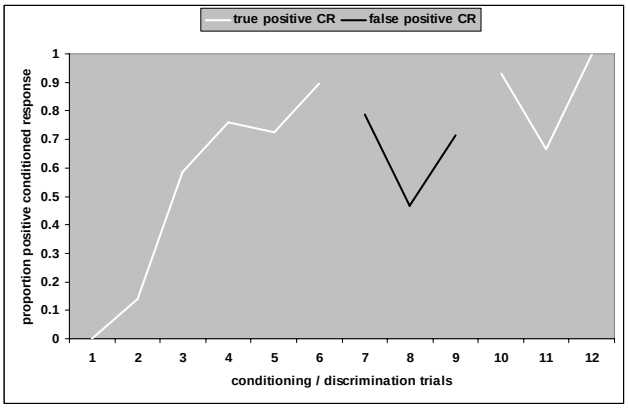

Figure 2: Conditioned responses (CR) exhibited by restrained honeybees to headspace from a grape leaf infected with powdery mildew (true positive) or an uninfected grape leaf (false positive) when subjected to a conventional acquisition protocol in which targets were delivered within a carrier gas of ambient air.

In honeybees subjected to the modified acquisition protocol, in which carrier gas contained headspace from an uninfected leaf, successful conditioning was also achieved, albeit to a lower degree: maximum positive CR was 37.9% during the fourth trial, decreasing to 17.2% during the sixth trial (Figure 3). During discrimination trials, 46.7-78.6% of previously-responding (n=26) bees subjected to conventional acquisition protocol and 0-20.0% of previously-responding (n=10) bees subjected to the modified acquisition protocol exhibited false positive CR when presented with a pulse of uninfected leaf headspace (trials 7-9, Figures 2, 3). After reintroducing and delivering the target (infected leaf) as the pulse, 66.7-100% of conventionally-conditioned bees and 30.0-40.0% of modified-protocol-conditioned bees exhibited true positive CR (trials 10-12, Figures 2, 3).

The asymptote of learning, evident through the maximum proportion of true positive CR exhibited, was much lower when using the modified acquisition protocol than when using the conventional protocol. This may be due to the fact that the continuous background in the modified protocol contained many of the same olfactory stimuli as the target, delivered as a pulse, therefore requiring more precise detection and discrimination. Learning ability in restrained preforaging honeybees has been associated with individual gustatory proclivity, or degree of food

motivation, and is genetically distinct from discriminatory ability (Scheiner et al, 2001).

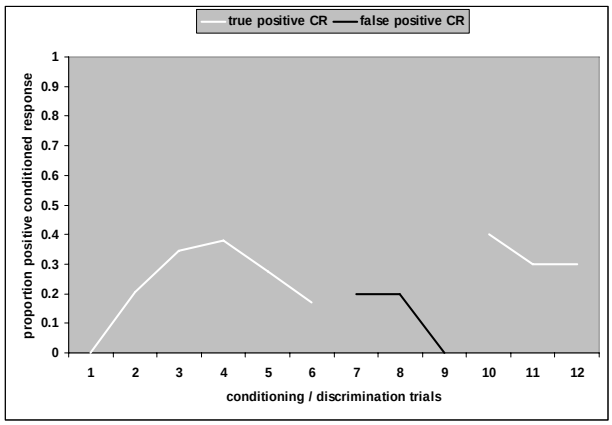

Figure 3: Conditioned responses (CR) exhibited by restrained honeybees to headspace from a grape leaf infected with powdery mildew (true positive) or an uninfected grape leaf (false positive) when subjected to a modified acquisition protocol in which targets were delivered within a carrier gas containing headspace from an uninfected leaf.

Accordingly, most bees exhibiting positive CR during conventional acquisition trials also exhibited (false) positive CR when exposed to headspace from an uninfected leaf, while a smaller proportion of previous responders during the modified protocol trials exhibited false positive responses. Perhaps the modified protocol selects for subjects with good discriminatory abilities during acquisition, while the conventional protocol selects for subjects with high gustatory proclivity and therefore high learning ability. In terms of biological sensor development, both high levels of learning ability and discriminatory ability are desired. Though clearly in its infancy, this work represents an important preliminary investigation into the feasibility of using PER observation as a biological sensor and indicator for early-season PM infections in commercial vineyards.

Acknowledgements

Bees were provided by UC Davis Entomology, facilitated by Susan Cobey and Michael P. Parrella. Plastics machining and equipment fabrication was in part provided by Tom Kominek and Richard Webb of UC Davis Plant Pathology. Much assistance and perseverance from Priyanka Parikh made data collection and analysis possible.

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Downy and powdery mildew spore monitoring in *Rioja Alavesa* vineyards, in the Northwest of Spain

A. M. Díez-Navajas, A. Ortiz-Barredo

NEIKER-Tecnalia. Department of Plant Production and Protection, Box 46. E-01080 Vitoria-Gasteiz, Spain.
e-mail address: adiez@neiker.net, aortizb@neiker.net

Introduction

Rioja Alavesa is a region situated in the South of Basque Country, in the Northwest of Spain (Figure 1). It is a wine producing area with 12,869 ha of vineyard, harvesting over 81,325,000 wine grape kilos. In this area, summer is dry and hot, reaching an average temperature over 22 ºC. Winter is quite cold, being favourable for frost and fog, but with little precipitation: less than 50 mm per month. With these climate characteristics, downy mildew, caused by the oomycete *Plasmopara viticola*, is rare, and generally occurs in shadowy areas and near rivers, where humidity is higher. In 2007, an outburst of downy mildew was detected after above-average precipitation during spring and summer. In contrast, powdery mildew, caused by *Erysiphe necator*, is present every year, and several treatments are applied to control it during the growing season. These applications are usually in based on a calendar schedule established by grape growers. Most of these applications are not necessary to control downy mildew.

Figure 1: Location of *Rioja Alavesa* in the Northwest of Spain.

We wanted to study the appearance and development of *E. necator* and *P. viticola* in vineyards of *Rioja Alavesa*, to obtain data for an agricultural warning service and thereby avoid unnecessary phytochemical applications, preserve environmental and applicator safety, and establish for each disease its risk assessment before spread. For this purpose, we installed 12 meteorological stations to monitor physical parameters involved in disease spread and evolution, and also installed spore traps to capture downy and powdery mildew spores at each location.

Materials and methods

Traps to capture airborne spores: To capture airborne spores of downy and powdery mildews, sticky glass traps were installed in supports. In each support four traps were placed, oriented towards the four compass points (Iturritxa and Ganley, 2007). Three supports were placed in each parcel, and traps in them at two heights from soil: 65 and 100 cm. Traps were replaced twice a week. They were taken to laboratory and observed unsing visible light microscopy, previously stained with acidic lactofucsin (0.1% lactofucsin in lactic acid). Spores for each mildew species were identified and the number reported for each sticky trap.

Meteorological stations: we installed 12 weather stations iMetos® (Pessl Instruments GmbH) in 12 different geographical locations to monitor environmental parameters corresponding to 12 vineyards. Each station was composed of several devices to measure various parameters: an anemometer for wind speed (m/sec), air and soil temperature sensors for underground and aerial temperatures(ºC), a relative humidity sensor for RH (%), a rain gauge for precipitation (mm), a solar radiation sensor (W/m2), a leaf wetness sensor (min), and tensiometers for soil matricial potential (cBar). Climate data downloading and disease risk models were provided by the MetwinII software of the iMetos ® station system. This software offers a risk model for downy and powdery mildew in grapevine. For the downy mildew risk model, it uses the parameters of precipitation, leaf wetness, air temperature and relative humidity. It is possible to obtain information about primary (sexual) and secondary (asexual) infections of the cycle. Air temperature and leaf wetness data are used for the powdery mildew risk model. Primarily based on the secondary cycle, the model also shows the date for possible infections by ascospores.

Results and discussion

We present only the results obtained for 4 locations. We evaluated the presence of downy mildew sporangia (asexual spores). For this, the weather station software uses the recorded data of precipitation, leaf wetness, air temperature and relative humidity to calculate a risk model. A lag of several days was observed from the first peak of spores up to the infection risk (Figure 2), i.e., the detection of spores occurring earlier than the calculated risk. The software determined powdery mildew risk using air temperature and leaf wetness. The spore peak for powdery mildew (Figure 3), the same as for downy mildew, was earlier than the slope of risk increased.

Spore counting can monitor spore occurrence, although favourable weather conditions must be present for the spores to be able to continue infection and development. Spore appearance can be used to validate the risk model for each area where stations were installed or to create a new one based in a pool of data taken during several growing seasons. We will eventually compare and assess the risk models offered by the weather stations installed in our parcels, with other models calibrated and validated on agrometeorological data collections (Orlandini *et al*, 2008) for sexual (Rossi *et al*, 2007) and asexual cycles, always compared to the presence or absence of spores until validation in our area.

Spore presence may reinforce information obtained from agrometeorological stations about the date in which disease risk was detected, validating obtained information from stations for the area where they were installed, and giving the possibility to use this information in an agricultural warning service. So advices informing about disease risks might be broadcasted, in order to apply phytochemicals treatments only when necessary,

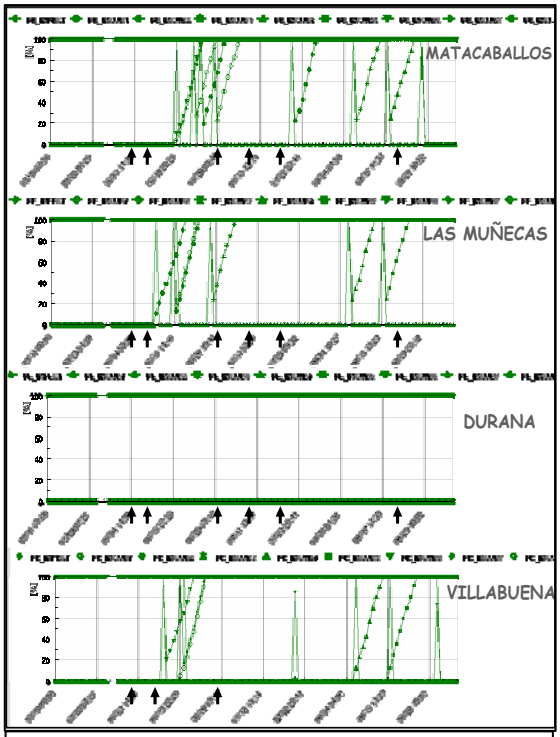

Figure 2: Risk model for downy mildew obtained from the weather stations in the parcels of Matacaballos, Las Muñecas, Durana and Villabuena. Arrows indicate a peak detection of spores. Graphics of downy mildew correspond to asexual cycle.

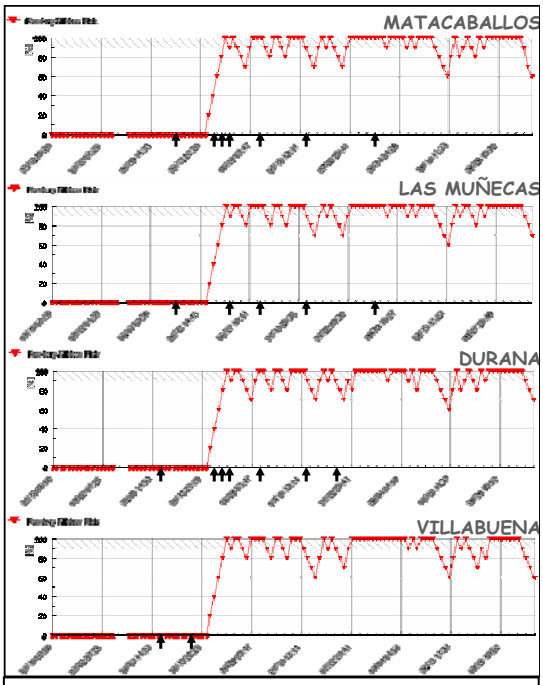

Figure 3: Risk model for powdery mildew obtained from the weather stations in the parcels of Matacaballos, Las Muñecas, Durana and Villabuena. Arrows indicate a peak detection of spores.

contributing in this way to reduce the phytochemical inputs into the environment.

The Directive 2009/128/EC of the European Parliament and of the Council establishes a framework for Community action to achieve the sustainable use of pesticides by reducing the risks and impacts of pesticide use on human health and the environment. It also promotes the use of integrated pest management and of alternative approaches or techniques such as non-chemical alternatives to pesticides. Consequently, we must study ways to reduce phytochemicals in vineyards, such as fungal spore monitoring to prevent unnecessary treatments.

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Effect of the grapevine growth on the dynamics of a powdery mildew epidemic: field trials and simulations

A. Calonnec, J. Jolivet, P Cartolaro, S. Schnee

INRA-Bordeaux, UMR INRA-ENITA 1065 Santé Végétale BP, 81, 33883 Villenave d'Ornon, France.

Introduction

The grape-powdery mildew pathosystem is characterised by a polycyclic pathogen capable of explosive multiplication, a host population with a high degree of spatial structure at the field level and with a complex architecture at the individual plant level exhibiting rapid changes over time. As well as environmental differences, the high degree of human interference during vine development and the wide diversity of cropping systems enhance variability from one crop to another. Furthermore, because of the tight relationship between powdery mildew and its host (Doster & Schnathorst, 1985, Gadoury *et al.,* 2003) and of the spatial location of primary infections on the vine stock, we hypothesized that the dynamic changes in crop structure should be considered as key factors for explaining variability in the severity of epidemic behaviour. The interactions between diseases and vine growth was observed in several studies dealing with the effects of crop practices on grapevine yield and quality (Evans *et al.,* 2006, Gadoury *et al.,* 2001, Intrieri *et al.,* 2001, Zahavi *et al.,* 2001). A characterization of the spatio-temporal spread of epidemics in the vineyard showed also higher velocity on plots with higher vegetative vigour (Calonnec *et al.,* 2009). Recently, an experiment showed that vigorous vines, grown with a high water and nitrogen supply, developed a higher number of diseased leaves and a higher percentage of mildewed berries compared to low vigour vines (Valdes, 2007). The major explanatory variable highlighted was the shoot leaf number, mainly early in the season. The study was, however, conducted on a cultivar moderately susceptible to powdery mildew (cv. Aranel). It was of prime importance to get data on more susceptible cultivars to see if the dynamic interactions are of the same magnitude, and how they could be exploited to better control the disease.

For a better understanding of these host/pathogen interactions and of the capacity of the host development to modify disease progress, we developed an epidemiological simulation model coupling vine growth with the dispersal and disease dynamics of *Erysiphe necator* (Calonnec *et al.,* 2008). The simulation model is a complex discrete deterministic model which incorporates explicitly the dynamics of host growth (distance between organs and their susceptibility) and the development and dispersion of the pathogen. Particularly, the model takes into account shoot topping which has for effect, to enhance the development of secondary shoots then the emergence of new susceptible leaves during the epidemic process. The flowering time is also a key period as the amount of disease at flowering is correlated to the damage on bunches on a susceptible cultivar such as Cabernet-sauvignon (Peyrard *et al.*, 2005, Calonnec *et al.*, 2006). It allowed simulating the spatio-temporal dynamics of host growth and epidemic development beginning from a range of climatic conditions, production systems and initial conditions for the density and location of the pathogen.

In order to assess if the plant could be considered as a key element of the protection system we examine the relationship between host and disease variables at key periods in the epidemic process, 1) in the field, after combining measures of vine and disease during an epidemic, and 2) in *silico*, after running simulations under different conditions of vine vigor and climatic scenarios.

Material and methods

Impact of host growth on a powdery mildew epidemic in the field. Experiments were conducted in plot, located on the INRA experimental field station (Domaine du Grand Parc, Latresne), with various levels of vigour generated by a combination of rootstocks (SO4, 110R and Riparia) and soil management (chemical weed control versus perennial cover crop). It is designed as 6 rows planted alternatively with two susceptible varieties Merlot and Cabernet-Sauvignon in 2001. The plot is shared in 8 blocks of 30 vines across the rows (6 rows x 5 vines). Each block is constituted by 6 sub-units combining each rootstock with each cultivar, randomly distributed. The first 4 blocks are conducted with perennial cover crop (CC) whereas the 4 others with weed chemical control (WC). On each sub-unit, one vinestock was selected based on its number of buds (7 for the Merlot and 8 for Cabernet-Sauvignon) and shoot's configuration. One shoot of this vinestock was inoculated at the stage "2 to 4 leaves" according to Calonnec *et al.* (2009). Several measurements were regularly made to characterize the vegetative growth of the vines. Two to three times per week, new emerged leaves were marked by colour markers and length of shoots was measured. The percentage of diseased foliar surface was estimated weekly on all leaves of the inoculated shoot and two of its neighbours. Nitrogen content in the soil is measured at the end of November with three samples per block.

In silico experiments. In order to identify favourable or unfavourable effects of crop growth, on the dynamics of the pathogen, we simulate epidemics using different environmental data and vine growth parameters that reflect:

- *3 contrasting seasons:* 2003 characterized by an early bud break (day 104) and an early flowering (day 152), 1998 a late bud break (day 114), late flowering (day 159), and 2004, later bud break (day 118) and later flowering (day 163) with an increased development rate (Figure 1). For simulations, the day of bud break and the day of flowering are achieved when the accumulated sum of the mean daily temperature above 10°C reaches 90 and 380 respectively starting from day 1 (1^{rst} of January). Shoot topping was simulated 10 days after flowering.
- *7 levels of vine vigour:* these levels result in an increased number and development of secondary shoots (Figure 1), especially after shoot topping.

Data analyses. The variables used to describe the host growth were: the number of leaves at flowering (NLflo),

the number of leaves at pea size berry stage (NLps), the number of leaves at the end of measurements (NLend), the rate of leaf appearance from first symptoms to shhot topping (RLE), the shoot length at flowering (StLflo) and the rate of shoot development (RSD). For the disease, the variables were: the number of diseased leaves at flowering (NDLflo), at pea size berry stage (NDLps), at the end of the measurements (NDLend), and the rate of diseased leaves appearance (RDLE). Phenological stages are presented in Table 1.

Table 1: Key periods of the vines development

Stage	Merlot	Cabernet-sauvignon
contamination	23 April	6 May
flowering	2 June	8 June
berry pea size	16 June	23 June
end of measurements	30 June	7 July

For the experiments *in silico,* four supplementary variables could be considered: the total or diseased leaf area at flowering, shoot topping or at the end of the season (SFlo, Sst, SDst, SD240).
PLS-path modelling analyses (Tenenhaus *et al.*, 2005) were performed to explore the relationships between host development, disease variables and the environment and to quantify the weight of each component. For the field experiments, the PLS-path model is described by 3 unobservable or latent variables (crop management, vine growth, disease). Each latent variable is constructed by a set of observable or manifest variables. The variable crop management is described by 1 quantitative variable: the soil nitrogen (N-sol), and 2 qualitative variables: the crop management (WC versus CC), and the rootstock (Pg-SO4, Pg-110R, Pg-R). The variables vine growth and disease are described by the manifest variables described above (NLflo, NLps, NLend, RLE, StLflo, RSD, NDLflo, NDLps, NDLend, RDLE).
For the simulations, the PLS-path model is described by 4 unobservable or latent variables (crop management, vine growth, years and disease). The three variables describing the "years" are the inverse of the sum of temperatures >10°C between bud break and flowering (1/ST°Bud-flo) or between flowering and the end of the season (1/ST°flo-240) and the date of bud break (Dbud). Vine growth and Disease are described by the variables described above (NLflo, NLps, NLend, RLE, StLflo, RSD, SFlo, Sst, SDst, SD240, NDLflo, NDLps, NDLend, RDLE). Finally, the crop management is described by the seven levels of vigour (Vig). The standardized latent variables are estimated as linear combinations of their centred manifest variables. The PLS path model is described by the measurement model relating the different manifest variables to their own latent variables and the structural model relating the endogenous LV "disease" to the other LVs: "vine growth" and "years". The entire model is important for determining the impact on the main target variable, the disease. The PLS-path modeling by using XSstat-Pro, module PLS-PM (Version 2010.2.02, Copyright Addinsoft 1995-2009).

Results

Effects of crop growth on the disease in the field. For Merlot (and Cabernet-Sauvignon, data not shown) the plant growth is characterized by an approximately linear development of primary leaves and a linear increase of leaf appearance on secondary shoots with strong variations between individuals at the end of the monitoring (Figure 2). The most vigorous vines presented three times more secondary leaves than the lowest vigor leaf. The number of diseased leaves follows an exponential curve, with an increase starting at the "flowering" stage. The last scoring date (68 days after contamination) shows an important variability of powdery mildew incidence on leaves.
According to the PLS path modelling analysis, the crop management has a significant effect on the Vine growth, for Merlot only but the correlation is weak (R^2=0.438 for Merlot, R^2=0.29for Cabernet which is not significant) (Figure 3). This could be explained by the high variability within individuals from the same soil zone (WC and CC) which indicate that the experimental design is not able to control sufficiently a vigor differential. Furthermore, the rootstocks have no significant effect on the vine growth. The disease is however well explained by the vine growth (CR2=69%) (R^2=0.79 for Merlot, R^2=0.87 for Cabernet). The disease variable is well described by NDLps, NDLend and RDLE. Individuals from the weed controlled blocks are characterized on average by a global increase of shoot development (SLf and RSD). NLps and NDPend show the highest correlation (R^2=0.953).

Effects of crop growth on the disease in silico experiments. From the simulations, an increase of the parameter of vigor from 0.2 to 1 amounted to a higher number of leaves at flowering (Nflo) and a higher rate of leaves emergence (RLE). The RLE was correlated with the number of diseased leaves at flowering (NDflo) and the rate of diseased leaves emergence (RDLE). An increase level of vigor has for consequence an increase level of disease surface area at shoot topping (SDst). The PLS-path scheme indicates that disease and vine growth are well described by their manifest variables except variables related to shoot development which are not significant (Figure 4). Vigor is the main contributor to the variation of vine growth (relative contribution =86.1%) compared with years (CR=13.8%). The disease is well correlated to vine growth (R^2=0.91, relative contribution=94.8%) through the indirect effects of vigor and years. The direct effect of years, through the temperature, on the disease is weak (relative contribution=5.2%). This means that in our simulations the main variability in the disease is due to the strong variations of vine growth mainly generated by vine vigor. The year has an effect on the dynamic of the severity of the disease with for example for the year 2004 with late bud break a higher level of the disease early in the season correlated to higher RLE.

Conclusions

The model strengthens experimental results observed regarding the effect of the rate of leaf emergence and of the number of leaves at flowering and pea size on the severity of the disease. However, the model underlines variation of the dynamics between years with possible variations on the damage. Experiments are undertaken to further explore the relationship between vine growth and disease development, 1) to demonstrate if disease development is only controlled by leaf number or also by variation in leaves susceptibility, 2) to quantify the year effect 3) to test which crop management could better control disease level.

Acknowledgements
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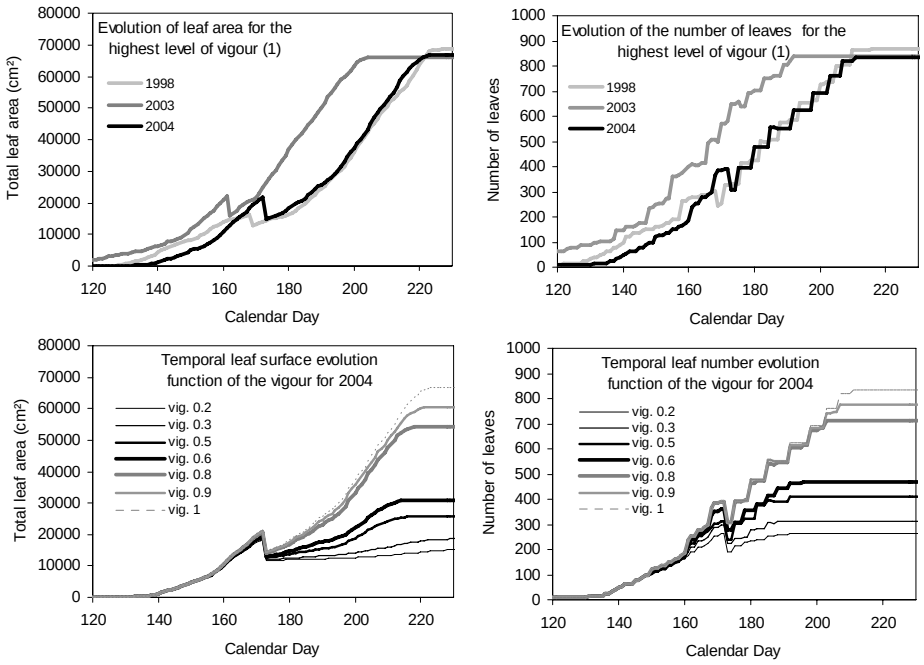

Figure 1: Comparison of the total leaf area and of the number of leaves per vine for simulations varying for the climatic conditions or for the vigour of the vine.

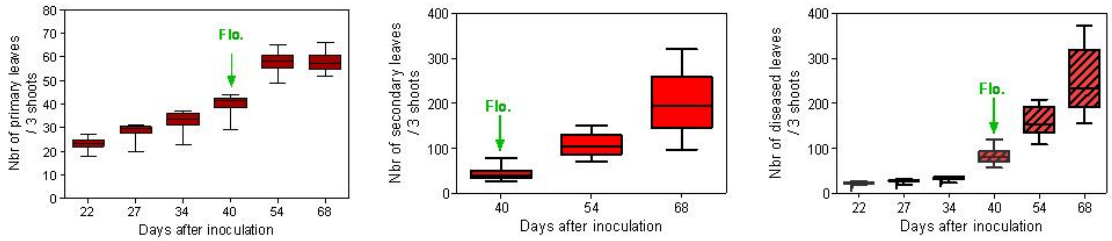

Figure 2: Distribution of the number of primary leaves, secondary leaves and diseased leaves observed on the 3 surveyed shoots on each selected vinestock function of day after inoculation for cultivar Merlot.

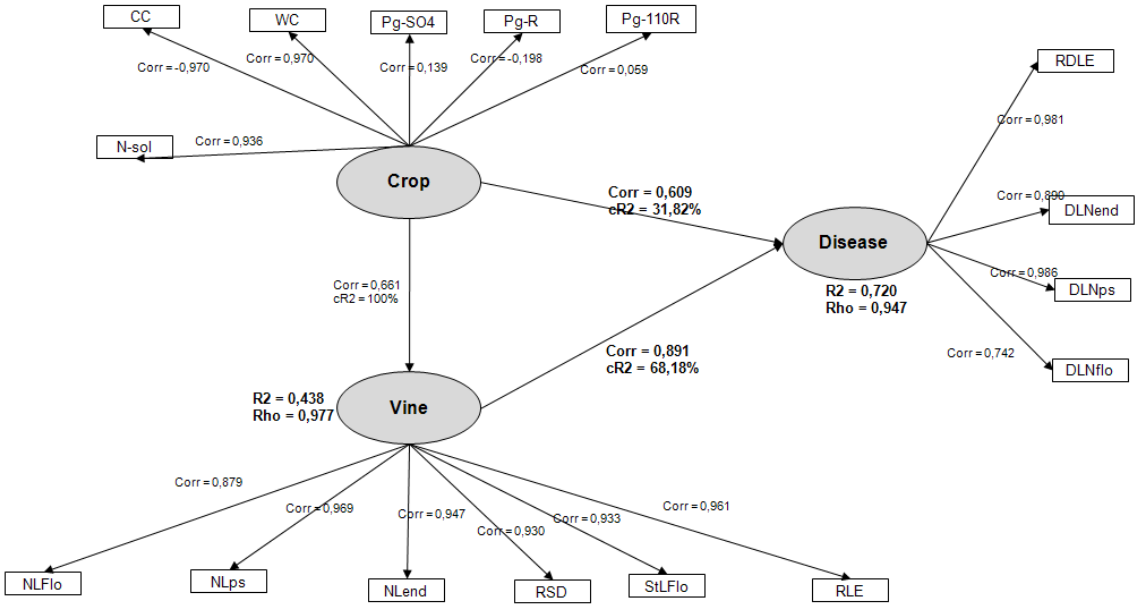

Figure 3: PLS path scheme based on field outputs for Merlot variety. The scheme describes the relationships between the latent endogenous variable "disease" to the other latent exogenous variables "vine growth" and "crop management". Corr, indicate the correlation coefficient between two latent variables with its confidence interval (Cl), CR2, the relative contribution of exogenous latent variables to endogenous one, R², the regression coefficient between exogenous and endonenous latent variables, Rho, the Dillon–Goldstein's coefficient.

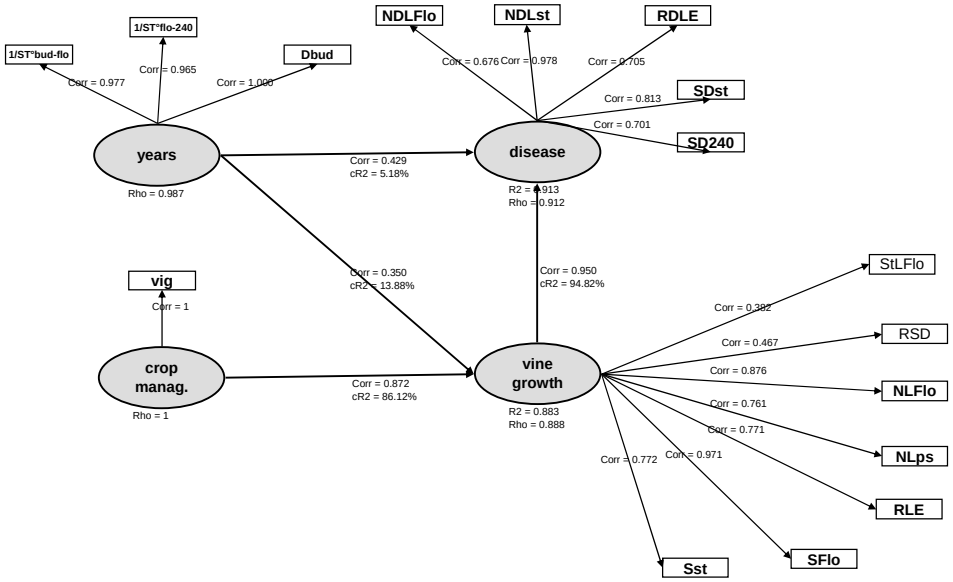

Figure 4: PLS path scheme based on simulations outputs. The scheme describes the relationships between the latent endogenous variable "disease" to the other latent exogenous variables "vine growth", "years" and "crop management".

Modelling the life cycle of *Erysiphe necator*

S.E. Legler[a], T. Caffi[a], V. Rossi[a], S. Giosuè[b]

[a]Università Cattolica del Sacro Cuore, Istituto di Entomologia e Patologia Vegetale, I-29122 Piacenza, Italy
[b]Horta Srl, spin off company of Università Cattolica del Sacro Cuore, I-29122 Piacenza, Italy

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The fungus *Erisyphe necator* (syn. *Uncinula necator*) (Schw.) Burr. is the causal agent of powdery mildew, a major grapevine disease throughout the world. Because this disease causes serious economic losses, the life cycle of the fungus has been extensively studied. *E. necator* is a polycyclic pathogen that exhibits polymorphism in its spore forms, with sexual (i.e., the ascospores) and asexual (i.e., the conidia) reproduction. Its life cycle is characterised by a chain of primary and secondary infection cycles that partially overlap in time (Figure 1).

E. necator overwinters as sexual fruiting bodies, the chasmothecia (formerly cleistothecia), on the vine bark (Pearson and Gadoury, 1987; Gadoury and Pearson, 1988; Cortesi *et al.*, 1997; Jailloux *et al.*, 1998; Füzi 1999). In spring, ascospores are repeatedly released from chasmothecia (Figure 2), and once they reach the leaf surface, they germinate and cause the primary infections though a biotrophic relationship with the epidermal leaf cells. The fungal colony grows on the leaf surface and forms the conidia, which cause new, secondary infection cycles and cause the epidemic development from late spring to summer (Gadoury and Pearson, 1988; Cortesi *et al.*, 1997; Gee *et al.*, 2000). In late summer, the pathogen forms new chasmothecia; because *E. necator* is heterothallic, these fruiting bodies form on the affected host tissue when two opposite mating types are in close proximity and their antheridium and oogonium combine (Schnathorst, 1965; Gadoury and Pearson, 1988). Mature chasmothecia can either be dispersed by rain splashes to the vine bark, where they overwinter, or they can release ascospores in autumn; the role of ascospore release in the late season is not clear. Increasing genetic diversity of the fungal population may increase the probability that two opposite mating types mate and, according to Gee *et al.* (2000), the infection efficiency of ascospores. In some cases, the pathogen can overwinter as mycelium in the dormant vine buds. Shoots developing in spring from affected buds are known as flag shoots, which represent a source of inoculum for secondary infections.

The processes described in the previous paragraph can be grouped into five stages: i) development and maturation of chasmothecia, ascospore dispersal, and ascospore infection; iii) growth of fungal colonies; iv) latency and clonal sporulation; and v) conidial infection. All five stages are strongly influenced by weather.

Control of powdery mildew is traditionally based on the management of secondary infections. According to a survey by the European Commission, in 2007 growers in Europe used 70000 tons of fungicides for grape protection, 53000 tons of which were used against *E. necator* (EC, 2007). In spite of this large use of fungicides, powdery mildew epidemics are frequently difficult to control because of the explosive nature of the infection cycles caused by clonal reproduction. Modelling plant diseases is a key approach for rationalizing disease management actions, including fungicide sprays (Rossi *et al.*, 2010). Weather-driven models have been developed for simulating parts of the life cycle of *E. necator* in order to evaluate the risk of powdery mildew infection and thereby to schedule fungicide applications. Some of these models are briefly described in the following paragraphs.

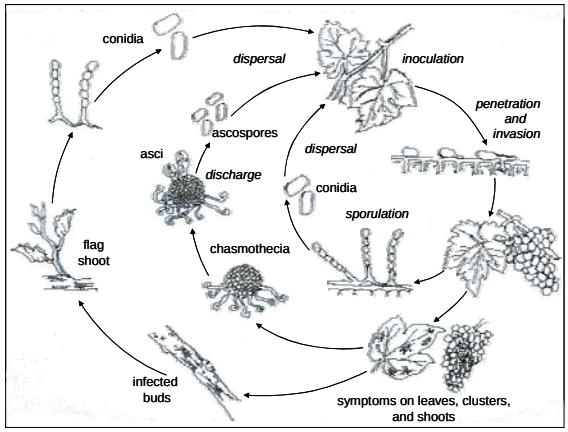

Figure 1: Life cycle of *Erysiphe necator*, the casual agent of powdery mildew of grapevine.

Models for chasmothecia development and maturation

Rossi *et al.* (2009a) developed a mechanistic dynamic model to predict the maturation of the *E. necator* chasmothecia in the vineyard. In this model, the chasmothecia advance from one stage of maturation to the next (i.e, from white, yellow, brown, and dark) at specific maturation rates that depend on air temperature Mature black chasmothecia are finally dispersed by rain splashes.

Models for primary infections or disease onset

Kast (1997) developed an empirical model for timing the first application of fungicides against powdery mildew based on data collected over more than 50 years in the wine regions of Wuerttemberg and Rheinhessen in Germany. This date is calculated as a time lag with respect to the development stage "three leaves unfolded", using a rough indexing of the disease severity for a vine site in the preceding year and the mean of the lowest temperatures in the two previous winters.

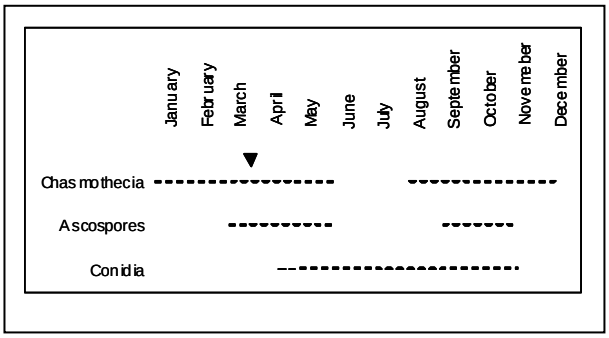

Figure 2: Presence of *Erysiphe necator* chasmothecia, ascospores, and conidia during the year in northern Italy. The arrow indicates the average time of grapevine bud break.

The UC Davis model (Gubler *et al.*, 1999) was developed for use in California. This is a rule-based model that accounts for both ascosporic and conidial stages of *E. necator*. The ascosporic part of the model was designed to estimate the risk of ascospore release from chasmothecia and consequent primary infection. Predictions are based on average temperature during an extended leaf-wetness event; the model uses the 'Conidial Mills Table' at 2/3s value for hours of leaf wetness required at various temperatures. In general, at least 12-15 hours of continuous leaf wetness are required when temperatures are between 10-15°C.

Gadoury and Pearson (1990) developed simple rules for minimal conditions for ascospore discharge from chasmothecia, i.e., 2.5 mm of rainfall and a temperature of 10°C.

Recently, Caffi and Rossi (2009) developed a mechanistic dynamic model for the simulation of *E. necator* ascosporic infections. The model uses air temperature, relative humidity, leaf wetness duration, rainfall, and vapour pressure deficit to calculate: i) ascospore maturation rates in spring; ii) ascospore dispersal events; iii) proportion of ascospores ejected in each discharge event; iv) infection efficiency of ascospores; v) probable onset of the disease symptoms; and v) duration of the latent period, i.e., the period between infection and production of asexual spores.

Models for secondary infections or disease risk

The model of Chellemi and Marois (1992) simulates the population growth of *E. necator* on *Vitis vinifera* 'Carignane' over time. This model follows the fate of each secondary infection cycle from germination of conidia until sporulation ceases; population size is determined by the number of viable conidia present each day. Equations accounting for the effect of temperature and liquid water on germination, penetration, and daily sporulation rates over the infectious period are included. The probability of conidia being deposited on susceptible leaf tissue is also considered.

Once ascosporic infection has occurred, the model of Gubler *et al.* (1999) switches to a risk index that is based entirely on the effect of temperature on the reproductive rate of the pathogen. The index fluctuates between 0 and 100; it increases by 20 points for each day with at least 6 hours between 21-30°C, while it decreases by 10 points for each day with less than 6 hours between 21-30°C or with a minimum temperature above 35°C. The use of the model allows grape growers to lengthen spray intervals during times of low to intermediate disease pressure and to shorten intervals when pressure is high. An index of 0-30 indicates that the pathogen is functioning minimally and is producing new conidia every 15 days or not at all. An index of 40-50 is considered normal and indicates that new conidia are being produced every 8-11 days. An index of 60-100 indicates the pathogen is producing new conidia every 5 days.

Kast (1997) developed a program named OiDiag that allows the grower to adjust the interval between sprays by calculating a temperature-dependent index accounting for powdery mildew development. Index values are calculated based on temperature, number of hours with humidity between 65 to 80% or >80% per day, duration of leaf wetness, and rainfall within periods of 14 days. The new version OiDiag-2.2 also considers the ontogenetic resistance of grapes in that higher index values are calculated for the period 10 days between the start of flowering and 10 days after the end of flowering.

Carisse *et al.* (2009) recently developed a risk assessment based on the relationship between incidence of powdery mildew on the leaves of different cultivars and cumulative concentration of airborne conidia in the vine production area of Quebec, Canada. An action threshold of 50 conidia per m^3 air per day is used for timing the interval between fungicide sprays.

Models for epidemic development

Sall (1980) developed a mathematical model accounting for the influence of seasonal weather patterns and timing of initial infections on fungus colonization of grapevine leaves and bunches. This model is mainly based on Vanderplank's equation; the powdery mildew infection rate is calculated based on temperature and moisture conditions. The model is linked to a vine growth submodel that describes the increase in the surface area of the susceptible parts of the vine, based on temperature.

Bendek (2002) developed a regression model to describe the development of powdery mildew incidence from flowering to the development of berries 5 mm in diameter in central Chile. The model is based on temperature and relative humidity.

Recently, Calonnec *et al.* (2008) developed a model coupling temporal and spatial vine growth with the development and spread of powdery mildew at the vine stock scale. In this model, the dynamics of the pathogen population on leaves are split into infection, colony or mycelium growth, and sporulation and dispersion. The time between infection and sporulation is described as the latent period, and the duration of sporulation is described as the infectious period. Temperature, wind speed, and wind direction are the main input variables. The environmental variables also dictate growth of the crop (appearance and growth of vine organs).

Despite the large number of relevant models, a holistic approach to quantitative modelling of the *E. necator* life cycle has been lacking. Recently, a model has been elaborated for *Plasmopara viticola* that links quantitative aspects of both sexual and asexual stages in a biologically coherent framework (Rossi *et al.*, 2009b).

An approach similar to the one used for *P. viticola* is proposed for *E. necator*, as diagrammed in Figure 3. This model considers the entire life cycle of the pathogen, allowing a global view of the pathosystem as a dynamic process. Rates of this model (i.e., discharge of ascospores, deposition of ascospores and conidia, infection by

ascospores and conidia, sporulation, mating, and maturation and dispersal of chasmothecia) and relevant periods (i.e., incubation, latency, and infectiousness) must be described mathematically as a function of the influencing weather variables. Several of these mathematical functions have already been elaborated in the previously cited models. This model should facilitate an integrated approach for protecting grapevines against powdery mildew and should guide all management options, from reducing the overwintering inoculum to protecting leaves and bunches.

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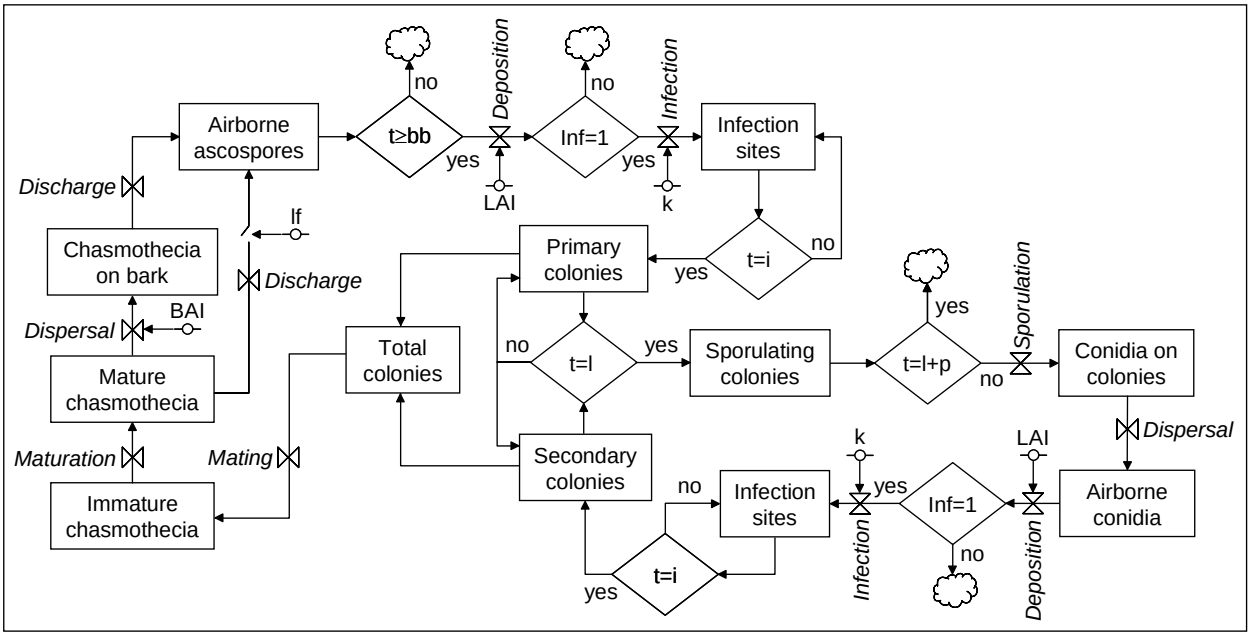

Figure 3: Relational diagram of a model describing the entire life cycle of *Erysiphe necator*. Boxes contain state variables, valves are rates, diamonds are switches, while clouds represent ascospores, colonies, or conidia that leave the system. Lines with circles are external variables related to the plant: LAI and BAI are leaf and bark area index, respectively; bb and lf are the time of bud break and complete leaf fall, respectively; k is the carrying capacity of the plant. The variable t is the day of the year; i, l, and p are incubation, latency, and infectious periods, respectively. Inf=1 means minimum conditions for infection are met.

Toward establishing low input regimes in Australian viticulture 2: Observations on the spatial movement of downy mildew, *Plasmopara viticola*, after a single secondary infection event in an South Australian vineyard

P.A. Magarey[a], T.J. Wicks[b]

[a]Magarey Plant Pathology, PO Box 220, Loxton, South Australia 5333 (formerly South Australian Research and Development Institute, Loxton, SA 5333) email: pmagarey@riverland.net.au.; [b]South Australian Research and Development Institute, Plant Research Centre, GPO Box 397, Adelaide South Australia 5001.

In discussing management strategies optimum for downy mildew, *Plasmopara viticola*, in South Australian vineyards, most grapegrowers and their technical advisers understand that the pathogen is readily dispersed by wind and rain. This leads to the thinking that downy mildew can spread for a kilometre or two and that, as a result, disease outbreaks in their vineyard could originate from inoculum in adjoining or more distant vineyards.

In devising optimum disease control strategies, the notion that a significant flow of inoculum from a neighbouring vineyard is invading 'my' vineyard, defers responsibility from 'me' to my neighbour. That is, from a location within my vineyard under my control, to the less tangible location 'beyond the boundary of my influence'. Such thinking is apt to detract from the principle that my disease management actions determine the level of downy mildew in my vineyard. This influence has been heightened during the recent significant increase in the number of abandoned vineyards in Australian viticulture, the result of a severe financial downturn in the profitability of production.

But what is the influence of 'my neighbour's' inoculum? How close does a vineyard with uncontrolled downy mildew need to be before inoculum levels influence the opportunity for successful control of the disease in an adjacent vineyard?

To answer some of these questions, observations on the spread of downy mildew were made following a temporally isolated infection event in a spatially isolated vineyard in the Barossa Valley, South Australia. This provided an ideal opportunity to map the spatial distribution of *P. viticola* from a single, naturally occurring secondary infection.

Source of inoculum. To establish a source of inoculum of *P. viticola*, T-trellised vines of *cv*. Biancone (syn. White Grenache), with a canopy ~2.0m x 2.0m x 1.5m, in an unsprayed vineyard at the SARDI Research Centre, near Nuriootpa, South Australia, were artificially inoculated with *P. viticola* on 14th November 1989. Five to 10 leaves/shoot on 5 shoots/vine on a single vine at the western end of each of three rows (Rows 2-4) were inoculated by hand-spraying the under-surface of each leaf with a sporangial suspension prior to sealing each subtending shoot in a plastic bag, as per Magarey *et al.* (1991a). The inoculated foliage was then incubated overnight at temperatures adequate for infection (Magarey *et al.* 1991b).

Spread of inoculum. Nine days later, on 23rd November, oilspots typical of *P. viticola* were seen on at least three leaves/shoot. After a further 6 days, on 29th November, a night time rainfall of 3mm with a minimum temperature 17^{0}C provided conditions suitable for an abundant sporulation and a significant secondary infection event in the vineyard (Magarey *et al.* 1991b). A prevailing westerly wind during that night led to a uniform distribution of sporangia downwind and a spread of inoculum in an elongate pattern away from the original cluster of infected vines.

On 7th December, twelve days after the secondary infection event, numerous new generation oilspots developed throughout the planting. Since no further rain or high humidity event was recorded prior to the event or during the incubation period that followed, the oilspots were considered a single cohort from infection derived from the upwind source of inoculum. This provided opportunity to measure the spatial movement of the disease and hence, assess the movement of sporangia from a point source of inoculum.

Assessing spread. On 14th December, the occurrence of oilspots in the vineyard was assessed by scoring the initially inoculated vine in each row and then every second vine downwind along the vine row. The incidence of infection was scored by estimating the % leaves with oilspots within a 0.25m^2 quadrat (with between from 30-40 leaves/quadrat) placed at random in the canopy of each vine. The severity of disease was determined by counting the number of oilspots/leaf on five leaves randomly selected in the same 0.25m^2 quadrat. These assessments were made on vines in the three rows with inoculated end-vines (Rows 2-4) and a similar array of vines in an adjacent uninoculated row (Row 1).

Results

The data from Rows 2-4 (Figure 1) showed that after the single secondary infection event, all leaves on each shoot on each inoculated vine had developed numerous oilspots.

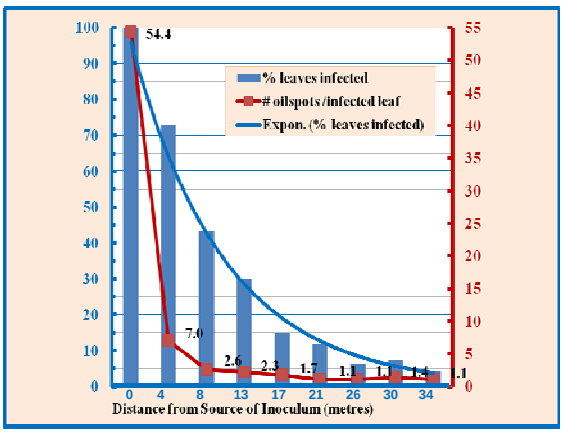

Figure 1: Spatial movement of inoculum of *Plasmopara viticola* during a single secondary infection in an unsprayed South Australian vineyard (Rows 2-4).

Four metres downwind, the incidence of oilspots was less though still very high (>70% leaves infected) but 8 m downwind, the incidence (43% leaves infected) was less than half that at the source. At 20 m from the inoculum source, the incidence was much lower (12% leaves infected). There was no further decline in incidence up to 34 m downwind on Rows 2 and 3 although on Row 4, 25 m from the inoculum source, there were no oilspots.

A similar but more dramatic decrease occurred in relation to the severity of disease – the initially inoculated vines showed an average of 54 oilspots/leaf, yet only 4 m downwind, there were 7 oilspots/infected leaf and between 20–34 m downwind, only ~1% leaves were infected. An isolated oilspot of the same cohort as the above, was detected in an adjacent unsprayed planting of *cv*. Crouchen vines ~300m downwind from the inoculated shoots.

Table 1 gives a spatial presentation of the disease gradient observed in Figure 1, but includes data from Row 1. The movement of inoculum, as assessed by disease incidence and severity measurements, conveyed the pathogen in abundance for a distance of 8 – 12 m and at low levels upward to 34 m. However, the disease did not move abundantly laterally to the south, across the direction of wind. Levels of oilspot movement from Row 2 to Row 1, ~ 3 m across the vine row, showed comparable levels of disease incidence as at 8 m downwind while severity was similar to that 4 m downwind.

Discussion

The pattern of spread and disease gradient we observed were consistent with the dispersion of disseminules from a point source observed by many authors (*e.g.* Gregory 1973). In our observations of downy mildew, an initial focus of infection led, through a single secondary infection, to a significant multiplication of disease. For example, an initial total of ~4,500 oilspots on the three inoculated vines resulted in 60,000 – 90,000 new generation oilspots and many more across the vineyard. The huge number of sporangia produced from the initial oilspots caused extreme levels of disease ~ 8-10 m downwind and less disease up to 34 m downwind. Disease incidence and severity at that distance were relatively minor compared to that in vines adjacent to the source of inoculum but were none-the-less significant in terms of providing a robust source of inoculum
for a second spread of disease if the conditions were to have favoured this.

The vineyard rows we assessed in this experiment did not extend beyond 34 m and thus prevented more detailed assessment beyond that range. However, the data presented gave indication that sufficient inoculum for low numbers of oilspots may have been dispersed a further 40 – 50 m but perhaps not more than 200 - 300 m beyond the row length we assessed. Our observation of the solitary oilspot in the *cv* Crouchen vineyard ~300m downwind, supports this assumption.

Summary conclusion

Field observations were made of the spatial distribution of grapevine downy mildew after a single, natural secondary infection event in a South Australian vineyard. We attempted to quantify in part, the well-known fact that the disease can spread rapidly and extensively in a vineyard as a result of a single favourable weather event. The incidence and severity of the pathogen increased from a point source of initial inoculum to a high level 8-12 m downwind while low levels reached at least 34 m downwind. Sporangia appeared to have moved in levels sufficient to need at least some management actions, 200 – 300 m from a point source. However, the data do not support the idea that inoculum spreads in significant titre much beyond these distances if any. In inland Australian viticulture, vine rows of just one cultivar, of the order of 500 m are not uncommon. Thus, in the main, the inoculum for initial generations of downy mildew disease in a vineyard can be assumed to originate principally from within 200-300 m of that location and not in some more distant vineyard. As a result, the responsibility for managing initial levels of inoculum lies with the manager of that vineyard and not with the managers of unsprayed neighbouring vineyards.

These data were consistent with observations on the spread of grapevine powdery mildew *Erysiphe necator,* in which for much of the season, the dispersion of that pathogen was over similar distances *i.e.* within-vineyard movement over 200-300 m. (Magarey and Emmett, *unpublished data*).

Our findings on the dispersion of downy mildew inoculum from a substantial secondary infection event, have relevance for vineyard managers when devising optimum management strategies for both this disease and for powdery mildew. This is especially true for vineyards near plantings abandoned during the recent downturn in Australian viticulture. The emphasis in managing early-season disease thus lies not with external sources of inoculum but with managing the inoculum that originates within the vineyard.

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Acknowledgement

The authors thank Malcolm Wachtel for assistance in collecting and assembling vineyard data during this project.

Table 2: Spatial distribution of oilspots of grapevine downy mildew after a single secondary infection event in an unsprayed South Australian vineyard (Rows 1-4). A westerly breeze persisted during the event.

Incidence - Estimated % leaves infected										**N ↑**
	Vine #	**1**	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**
Row #	**4**	**100**	**75**	**20**	**15**	**10**	**10**	**0**	**-**	**-**
	3	**100**	**64**	**60**	**50**	**20**	**10**	**10**	**12**	**10**
	2	**100**	**80**	**50**	**25**	**15**	**15**	**9**	**10**	**3**
	1	**50**	**50**	**20**	**40**	**5**	**0**	**0**	**0**	**0**
Severity - Mean # oilspots /infected leaf										
	Vine #	**1**	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**
Row #	**4**	**45.2**	**4.6**	**1.6**	**2**	**1.8**	**1**	**0**	**-**	**-**
	3	**71.2**	**5.8**	**2.8**	**2.8**	**1.4**	**1.4**	**2**	**1.6**	**1.2**
	2	**46.8**	**10.6**	**3.4**	**2**	**2**	**1**	**1.4**	**1.2**	**1**
	1	**9.4**	**2.4**	**1**	**1.2**	**1.4**	**0**	**0**	**0**	**0**
Distance from Source of Inoculum (metres) for rows 2-3-4		**0**	**4.3**	**8.5**	**12.8**	**17.1**	**21.3**	**25.6**	**29.9**	**34.1**

Can cultivar mixtures in organic vine growing reduce downy mildew severity?

C. Matasci[a] , M. Jermini[b], C. Gessler[a]

[a]Plant Pathology, Institute of Integrative Biology, ETH-Zürich, Switzerland; [b]Research station Agroscope Changins – Wädenswil ACW, Centre of Cadenazzo, CH-6594 Contone, Switzerland

In northern rainy regions copper is often not sufficient and strong attacks can result in complete plant defoliation and production of low quality or entirely destroyed grapes. Moreover copper has deleterious effects on living organisms in soil and therefore the permitted amounts are limited. Positive effects (reduction of disease severity) of increasing the functional biodiversity in terms of crop mixtures or cultivar mixtures opposed to monoculture are reported in literature for different crops.

Material

We established a vineyard consisting of eight *Vitis vinifera* and hybrids cultivars differing in resistance/susceptibility to *Plasmopara viticola* (Table 1) arranged in four within-row mixture blocks and in one one-cultivar-by-row block (Figure 1).

Result and Discussion

The three years of epidemiological observations in the experimental plot differed significantly Figure 2). In the first year (2005), the lowest *P. viticola* disease severity and incidence levels were assessed, in the second year (2006) the highest values were observed. While in 2007, year in which meteorological conditions were most disease conducive and two fungicide applications were performed to slow down the epidemic, intermediate disease levels were assessed.

Levels of *P. viticola* disease on the eight *V. vinifera* and interspecific hybrid cultivars in the three years corresponded to the assumed ranking in respect to *P. viticola* resistance (Jermini, personal communication). High disease severity and incidence were observed on the highly susceptible Müller-Thurgau and the susceptible cultivars Gamaret and Merlot. Moderate disease for the less susceptible Isabella, and low disease for the resistant Regent and Bianca and the highly resistant cultivars Solaris and Chambourcin.

In the first year the planting system caused clear and significant differences when comparing the performance of each cultivar in the two systems: lower disease severity and incidence (not statistically significant in any date for Gamaret and Merlot) was assessed in the MIX block for the susceptible cultivars Müller-Thurgau, Gamaret, Merlot and Isabella, while lower disease severity and incidence was assessed in the MONO block for the resistant cultivars Regent, Bianca, Solaris and Chambourcin. Disease reduction in mixtures operates in three principal ways: (i) dilution of inoculum, (ii) physical barrier effect and (iii) induction of defense reactions in the host. The first two mechanisms occur with high probability for the susceptible cultivars in our plot, if also the third one contributes can not be determined by our data. Andrivon *et al.* (2003) observed that potato cultivar mixtures decrease the *P. infestans* epidemic spread on the susceptible cultivar without altering the behaviour of the partially resistant one(s). In our experiment we observed an increase of disease on the more resistant cultivars in mixture; this could depend from a higher disease pressure exerted by the presence of susceptible vines interposed among the more resistant ones.

The trend for lower disease in the MIX block than in the MONO block for susceptible cultivars was observed again in 2007 (Müller-Thurgau and Gamaret). This strong decrease in mixture efficacy in reducing disease on susceptible cultivars could depend from the more conducive conditions observed in 2006 and 2007. This would be in accord with Andrivon *et al.* (2003) who observed for the potato-*Phytophthora* infestans pathosystem that progress rates were reduced and sometimes delayed in mixed plots compared to unmixed ones only for the slowest epidemic. Oppositely Leonard (1969) indicates that disease reduction due to mixing will increase with increasing generations of pathogen reproduction. Cowger and Mundt (2002) observed that in a year with severe epidemic, mixtures of wheat cultivars were less diseased than were their component pure stands. The following year the epidemic was mild, mixtures did not reduce nor increase *Mycosphaerella graminicola* disease severity, the third year the epidemic intensity was intermediate, and mixture plots were more diseased than the mean of component pure stands. Mundt (2002) concluded that "if high disease incidence in pure stands is due to a fast approach to carrying capacity or large amounts of outside inoculum (either initially or on a continuing basis), then mixtures may be less effective in severe than in less severe epidemics. If, on the other hand, high disease incidence in pure stands is being driven by the number of pathogen generations, then mixtures may be more effective in severe than in less severe epidemics. *P. viticola* epidemics are characterized by a continuous contribution of primary oosporic infections throughout the season (May to late October, depending on the region), with most genotypes having a limited ability to spread asexually (Gobbin *et al.* 2003, Rumbou and Gessler 2004, 2006), this could partially explain the decrease in mixture efficacy in reducing *P. viticola* observed in 2006 and 2007 in our experiment. However this is probably not the only explanation, in so far as in 2007 two copper treatments were applied the 12th and 28th of June reducing disease and slowing down substantially the epidemic. An adaptation of *P. viticola* to one or more components of the mixture could be an ulterior explanation.

Results from the genetic analysis. *Plasmopara viticola* lesions were collected in 2005 and 2006 in the mixed grapevine cultivar plot composed from vines of the eight cultivars. No difference in genetic structure of *P. viticola* populations depending on different spatial aggregations of grapevines (MIX block or MONO block) was observed in 2005 and 2006. In both years significantly lower genotypic diversity was observed on populations collected on resistant cultivars, while higher diversity was observed for the ones collected on susceptible vines. For 2006 pathogen adaptation leading to erosion of partial resistance can be

hypothesized occurring on the three related cultivars Regent, Bianca and Chambourcin. Further adaptation to the dominant resource can have occurred in the plot so as generally over years in the vineyards for the most frequent cultivars Merlot, Gamaret and Isabella-like American red vines.

Conclusions

Considering the low or absent positive effect of mixtures in reducing *P. viticola* disease in the second and third year of observations, so as the difficulties of planting, growing, harvesting and handling that a mixed cultivar will imply, we can suppose that grapevine cultivars mixture are not an effective method to reduce *P. viticola* disease in vineyards and therefore reduce the amount of copper applications in viticulture.

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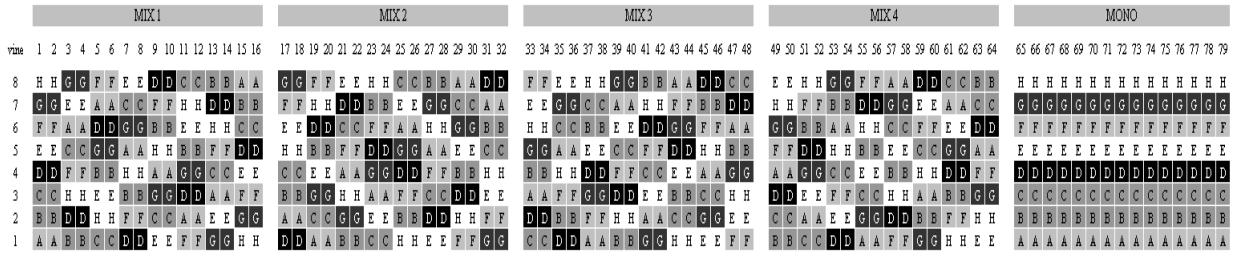


Figure 1: Representation of planting arrangements of the eight *V. vinifera* and interspecific hybrid cultivars in the experimental plot. In the first four blocks grapevine cultivars are arranged in a within-row mixture (MIX 1 to 4), in the fifth block vines of each cultivar are arranged in one one-cultivar-by-row system (MONO). Each square represents one vine, letters from A to H represent the eight *V. vinifera* and interspecific hybrid cultivars (A: Regent, B: Merlot, C: Isabella, D: Müller-Thurgau, E: Solaris, F: Bianca, G: Gamaret, H: Chambourcin), grey intensities represent levels of resistance (black, highly susceptible cultivar (– –); dark grey, susceptible cultivar (–); light grey, less susceptible cultivar (–/+); lighter grey, resistant cultivar (++); white, highly resistant cultivar (+++), in brackets code used in Tab. 1).

Table 1: Parentage, country of origin, year of crossing and level of resistance, surface and percentage of the whole viticultural area in Ticino (Southern Part of Switzerland) for the cultivars used in the experiment.

Cultivars	Parentage[a]	Country of origin[a]	Year of crossing[a]	Level of resistance[b]	Surface (ha)[c]
Müller-Thurgau[d]	Riesling × Madeleine Royale	D	1882	– –	2.47 (0.24%)
Gamaret[d]	Gamay × Reichensteiner	CH	1970[f]	–	18.49 (1.78%)
Merlot[d]		F		–/ +	834.12 (80.37%)
Isabella[e]	*V. labrusca* × *V. vinifera*	USA	1816	–/ +	1.32 (0.13%)
Regent[e]	Diana × Chambourcin	D	1967	+	1.61 (0.15%)
Bianca[e]	Eger 2 × Bouvier	HUN	1963	+	0.09 (0.01%)
Solaris[e]	Merzling × (Saperavi severnyi × Muscat ottonel)	D	1975	++	0.91 (0.09%)
Chambourcin[e]	Seyve Villard 12-417 × Chancellor	F		++	2.31 (0.22%)

[a] From VIVC 2007
[b] – – , highly susceptible cultivar – susceptible cultivar, –/ + less susceptible cultivar, +, resistant cultivar, ++, highly resistant cultivar (Jermini, personal communication)
[c] From DFE 2007
[d] *Vitis vinifera* cultivar
[e] Interspecific hybrid
[f] From Basler and Pfenninger, 2002

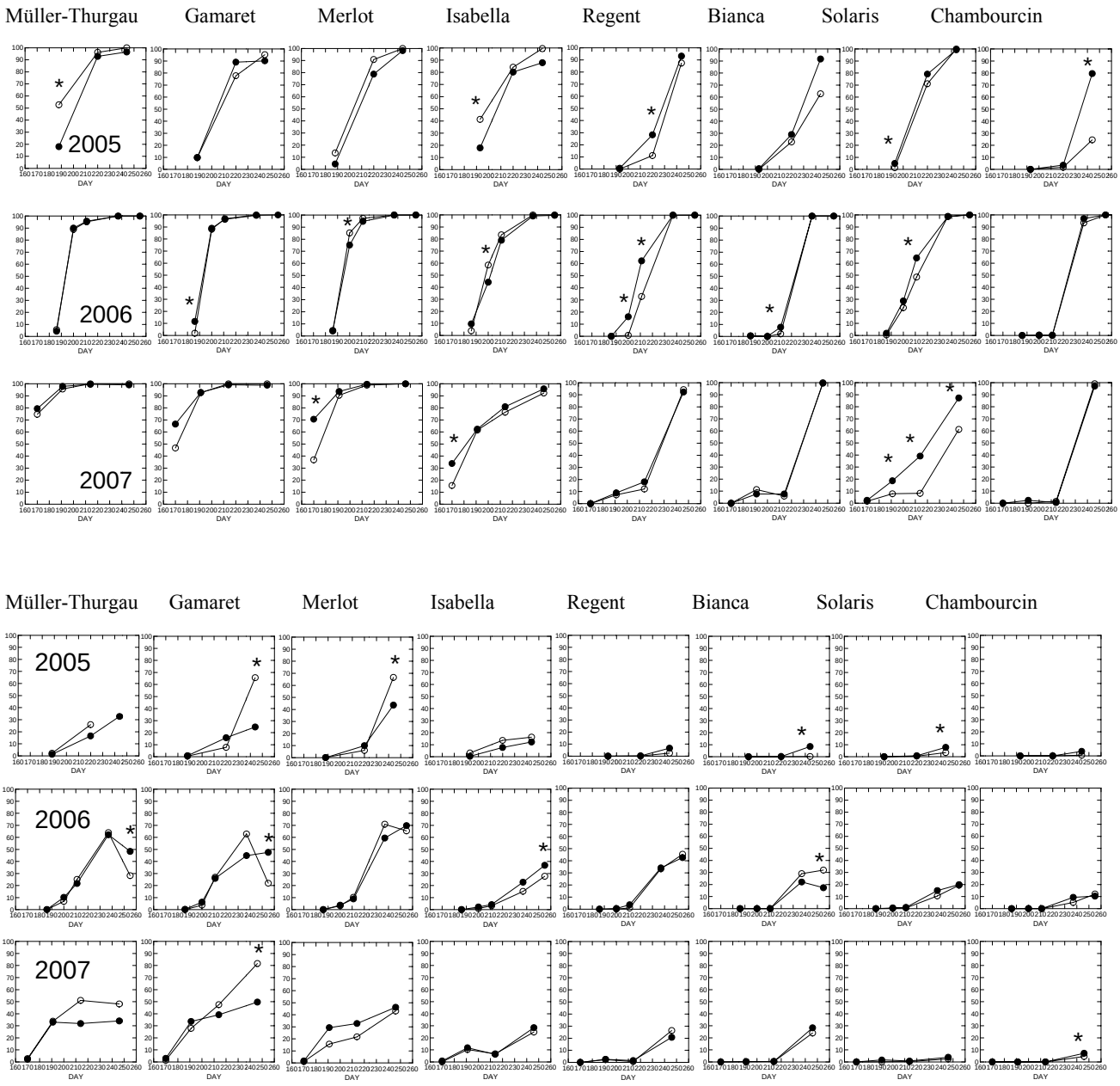


Figure 2: Evolution of *P. viticola* incidence (upper part) and severity (lower part) in the MIX (black circle) and in the MONO block (white circle) assessed on primary shoots of vines of cultivars Müller-Thurgau, Gamaret, Merlot, Isabella, Regent, Bianca, Solaris and Chambourcin. Cultivar susceptibility decreases from left to right. Data from 2005 (first line), 2006 (second line) and 2007 (third line). Values on the y-axis indicate % disease incidence/severity. Days indicate Julian days. Each point represents the mean of the incidence assessed on the primary shoot of 72 (four MIX blocks) respectively 15 vines (one monoblock). Exceptions: 2005: by the first assessment 32 vines in the MIX (four blocks) and 8 vines in the MONO, by the second and third assessment 16 vines in the MIX (four blocks) and 8 in the MONO; 2006 and 2007: by the first assessment 16 vines in the MIX (one block) and 15 in the MONO. Asterisks indicate significant differences ($P \leq 0.05$) between values assessed on vines in MIX and in the MONO block system

Early symptoms assessment as indicator to control Grapevine Powdery Mildew with reduced fungicide applications

P. Cartolaro[a], L. Delière[a], L. Delbac[a], O. Naud[b], A. Calonnec[a]

[a]INRA-Bordeaux UMR INRA-ENITA 1065 Santé Végétale – ISVV, BP81, 33883 Villenave d'Ornon – France, e-mail cartolaro@bordeaux.inra.fr, [b] Cemagref – UMR ITAP, BP 5095, 34196 Montpellier Cedex 5 – France.

In French vineyards, epidemics of powdery mildew *(Erisyphe necator)* occur with a high variability within years and places. Damage caused by the disease can be severe as in 2004 in Bordeaux on Merlot cultivar or in Champagne and Burgundy on Chardonnay. Except in the South region and mainly on Carignan cultivar, flagshoots are rarely or never observed in most of the French vineyards, and therefore are not responsible for the initiation of epidemics. Conversely, cleistothecia are frequently observed on leaves after harvest and ascospores can be released at spring as described in several countries (Cortesi *et al.,* 1997, Gadoury *et al.,* 1988. Jailloux *et al.,* 1998, Pearson and Gadoury 1987). Primary symptoms are observed on leaves located first or second rank from the base of shoots, near the bark of the trunks (photo). Moreover, early foci cause higher epidemic development on leaves and greatest damage on bunches (Calonnec *et al*., 2006)

No forecast model is actually useful in France to drive strategies of protection (except in flagshoot cases in the South where initiation of the disease is well known) and usual preventive management is based on growth stages of the vine. Therefore, early detection of symptoms seems the actual most appropriate way to validate the presence of early disease.

Our study conducted from 2003 to 2009 in commercial vineyard conditions, had the objective to evaluate powdery mildew [pm] decision rules based on: (i) incidence disease assessments early in the season, giving so the starting time of the epidemic; (ii) a few number of well-timed fungicide applications according to the critical times of the epidemics development (increase phase of disease incidence on leaves before flowering and highest risk period for young berries infection at flowering) (Gadoury *et al.,* 2003).

Material and methods

The study is conducted in two steps. A preliminary experimentation had been realised in the Bordeaux vineyard from 2003 to 2007, to adjust and validate the decision rules. Since 2008, the experiment is conducted within national network in collaboration with extension services and vinegrowers.

Description of the pm rules

- A first pm rule applied in 2003 and 2004 was based on two systematic treatments, at flowering stage (BBCH-67) (T3) and 14 days later (around BBCH-75) (T4), and two optional treatments depending on the results of two disease assessments: first one (T2) at pre-flowering stage (BBCH-57) if I_1>5% and second one (T5) at bunch closure (BBCH-77) if I_2>10% (Table 1). Following this rule, a minimum of 2 treatments up to 4 can be applied.

Table 1: Description of the decision rule applied in 2003 and 2004

Growth stage[(a)]	Indicator			Fungicide application	
	Type	Sampling		Type	Condition
		plants	organs/plant		
15/17	**I_1** : incidence of dis. plants	1/10	4 leaves[(b)]	-	-
57	-	-	-	Optional (T2)	**I_1** >5%
67	-	-	-	**Systematic (T3)**	-
75	-	-	-	**systematic[(c)] (T4)**	-
77	**I_2** : incidence of dis. bunches	1/10	5 bunches	Optional (T5)	**I_2** >10%

(a) according to BBCH scale **(b)** located on 1st or 2nd rank from the base of the shoot
(c) 14 days after the previous application

Table 2: Description of the decision rule applied from 2005.

Growth stage[(a)]	Indicator			Fungicide application	
	Type	Sampling		Type	Condition
		plants	organs/plant		
15/17	**I_{1a}**: incidence of dis. plants	1/10	4 leaves [(b)]	**Systematic (T1)**	-
57	**I_{1b}**: incidence of dis. plants	1/10	6 leaves [(c)]	Optional (T2)	**I_{1b}** >10%
67	-	-	-	**Systematic (T3)**	-
75	-	-	-	Optional [(d)] (T4)	**I_{1a}** > 2% or **I_{1b}** >10%
77	**I_2** : incidence of dis. bunches	1/10	5 bunches	Optional (T5)	**I_2** >20%

(a) according to BBCH scale **(b)** located on 1st or 2nd rank from the base of the shoot
(c) located on 4th to 7th rank from the base of the shoot **(d)** 14 days > previous spray

- In 2005, the pm rule was modified to improve the control of the disease mainly in the early phase of the epidemic development: the first systematic treatment being applied earlier at pre-flowering stage (BBCH 15/17) (T1) and the second one at flowering (T3) (Table 2). A second assessment I_{1b} was added at stage BBCH 57. Thus, an optional treatment (T2) could be applied before flowering in case of high level of disease assessed on leaves (I_{1b}>10%) around two weeks after the first assessment and treatment. A second one (T4), after flowering, was applied at stage 75 [BBCH scale] depending on the level of disease at pre-flowering (I_{1a}>2% or I_{1b}>10%), and the last treatment (T5) at bunch closure if more than 20% of the bunches were diseased at

stage 77 (third and last decisional assessment I_2). Thus, according to this pm rule, a minimum of 2 treatments can be applied in case of none or low disease level, up to 5 if high level of disease is assessed in the parcel.

Experimental design

- Commercial parcels of 0.25 to 2 ha, without replicates and untreated plots, under natural disease infections.
- Disease assessments are realised in a central area of the parcel of 1000 plants (whatever the size of the parcel and planting density), on each site:
  - to evaluate decisional indicators, the frequency of diseased plants on leaves or diseased clusters is assessed over 100 plants (10% sampled);
  - to evaluate the strategy efficacy, the average severity of all the bunches of 30 sampled plants is assessed at pre-harvest stage (beginning of colour change).
- Fungicides applied are chosen indifferently among DMI and Strobilurin groups and all treatments are performed by the growers with their usual equipments.
- Sites and vine cultivars. Since 2003, the experiment is conducted in INRA commercial vineyards near Bordeaux, in 2 sites (Latresne – Cadaujac) with different disease history, less than 10 km away from one another, both on merlot and cabernet-sauvignon cultivars. In 2007, three parcels of private vineyards were added, on merlot (2 sites) and cabernet-franc (1 site). Since 2008, experiment is extended within national network including South and further East regions of the French vineyards with local representative cultivars: 22 parcels in 2008 and 37 in 2009 (Table 3).

Table 3: Number and geographical repartition of parcels and cultivars where the powdery mildew decisions rules are applied from 2003 to 2009.

	Year	Region	Vineyard	Cultivars (nb of sites)	Total parcels
Preliminary study (1st rule)	**2003**	West	Bordeaux	Merlot (2)	2
	2004			Merlot (2)	2
	2005	West	Bordeaux	Merlot (2) Cab-Sauvignon (2)	4
	2006	West	Bordeaux	Merlot (2) Cab-Sauvignon (2)	4
	2007	West	Bordeaux	Merlot (4) Cab-Sauvignon (2) Cab-Franc (1)	7
National network	**2008**	West	Bordeaux	Merlot (8) Cab-Sauvignon (3) Cab-Franc (1)	12
		South	Languedoc-Roussillon	Carignan (3) Syrah (2) Mourvèdre (1) Merlot (3) Chardonnay (1)	10
	2009	West	Bordeaux	Merlot (10) Cab-Sauvignon (4) Cab-Franc (1) Sémillon (1)	17
			Cognac	Ugni blanc (1)	
		South	Languedoc-Roussillon & Côtes du Rhône	Carignan (4) Syrah (1) Mourvèdre (1) Grenache (2) Merlot (3) Chardonnay (1)	12
		East	Beaujolais & Mâconnais	Gamay (4) Chardonnay (2)	8
			Jura	Trousseau (1)	
			Champagne	Pinot noir (1)	

Results

Preliminary study from 2003 to 2007

- *First pm decision rule.*

In the Bordeaux vineyards, powdery mildew epidemics were moderate in 2003 and much higher in 2004, with high variability between sites. In the Cadaujac merlot parcel, none symptom was observed on leaves at early stage first year (I_{1a} assessment) as well as at closure stage on bunches. According to the first rule, only the 2 systematic treatments were applied at flowering (T3, T4), providing well protected berries at harvest (Table 4). On the other hand, near of 20% of plants showed early symptoms in Latresne merlot parcel, triggering the first optional treatment at pre-flowering (T1). With the 2 systematic treatments, this strategy achieved good control of the disease.

In 2004, 2% of diseased plants were assessed at I_{1a} in the Cadaujac parcel, and T1 treatment was not triggered. The two systematic treatments at flowering didn't achieve control of berries infection and the optional treatment T5 was applied at closure after the I_2 assessment on bunches (35%). This gave satisfactory protection at harvest with 0.4% severity of powdery mildew on bunches in spite of 21.6% disease incidence (one or two infected berries / diseased bunch). In Latresne parcel, higher powdery mildew epidemic was observed; I_{1a} and I_2 indicators showed 23.5% and 70.4% disease incidence, respectively on plants (leaves) and on bunches. The full 4 treatments program applied resulted in 63% incidence and 2.3% severity which is at the limit of acceptability. These results showed that pre-flowering control of the disease on leaves have to be reinforced to assure better control of berries infection at flowering in case of high epidemic.

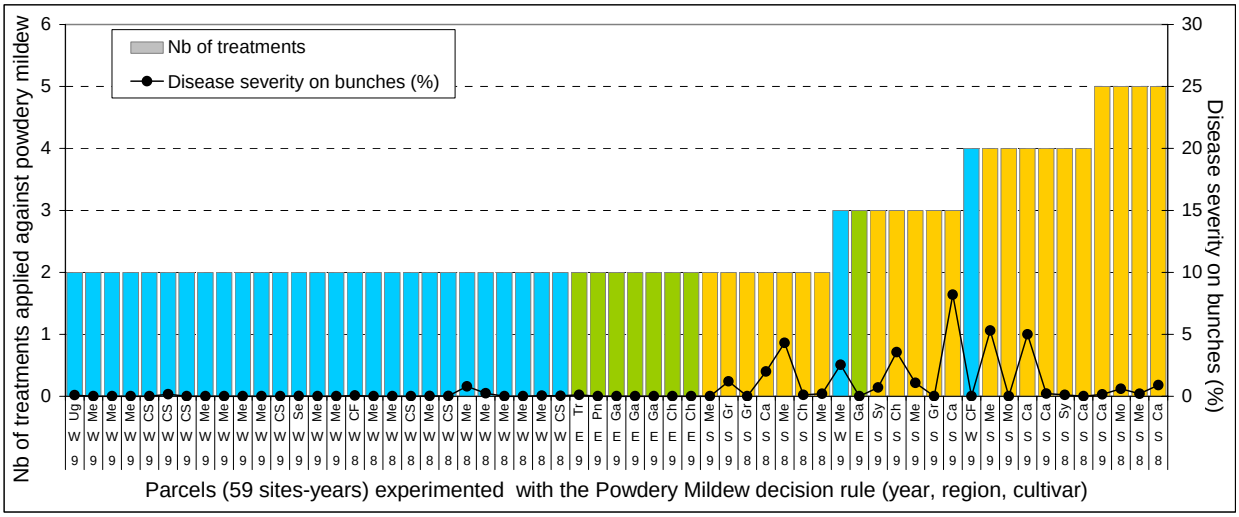
- *Second pm decision rule.*

In 2005, the second rule was applied in Cadaujac and Latresne sites, in merlot and cabernet-sauvignon parcels. Epidemic level was generally lower than the previous year in the Bordeaux vineyard. In Cadaujac site where early disease level was low, grapes were well protected (<0.1% severity on bunches) applying 2 treatments (T1-T3), on both the merlot and cabernet–sauvignon parcels. In Latresne site, early disease level was higher, mainly in the merlot parcel (I_{1a} = 14.4%). Despite systematic treatment T1 applied, powdery mildew continued increasing on leaves (I_{1b} = 26.8%) triggering the two optional treatments T2 and T4. With the systematic treatment T3 applied, this led to reduce berries infection under the 20% incidence threshold of I_2, without T5 treatment application. This strategy achieved satisfactory protection with 0.6% severity on bunches at harvest. Again, successful powdery mildew control was achieved in cabernet-sauvignon parcel with 2 systematic treatments T1-T3.

In 2006, and further in 2007, powdery mildew level was lower. The two treatments program (T1-T3) was successfully applied in all the experimented parcels, except for Latresne merlot parcel where respectively 4 and 3 treatments were realised according to the presence of early symptoms. In 2007, the second disease assessment (I_{1b}=7.4%) was under the 10% threshold to trigger the T2 treatment, leading to 2.7% severity at harvest. It seems that it would have been useful to apply this treatment to achieve good final control as reached in 2006.

Table 4: decisional indicators values, treatments applied and disease assessment at harvest (incidence and severity) on bunches.

year	disease level in vineyards	site	cultivar	decision rule applied	disease level indicators			fungicide treatments						bunches infestation at harvest	
					on leaves before flowering (% of plants diseased)		on bunches before closure (% of bunches dis.)	T1	T2	T3	T4	T5	Total Nb		
					I1a	I1b	I2							Incidence	Severity
2003	-/+	*Cadaujac*	*merlot*	*1st*	*0.0%*		*0.0%*			*X*	*X*		*2*	*0.003%*	*< 0.1%*
		Latresne	*merlot*	*1st*	*19.9%*		*0.9%*	*X*		*X*	*X*		*3*	*1.1%*	*< 0.1%*
2004	++	*Cadaujac*	*merlot*	*1st*	*2.0%*		*35.0%*			*X*	*X*	*X*	*3*	***21.6%***	*0.4%*
		Latresne	*merlot*	*1st*	*23.5%*		*70.4%*	*X*		*X*	*X*	*X*	*4*	***62.9%***	*2.3%*
2005	-/+	Cadaujac	merlot	2nd	0.6%	1.2%	0.4%	X		X			2	1.4%	< 0.1%
			cab-sauv.	2nd	0.0%	0.0%	0.9%	X		X			2		< 0.1%
		Latresne	merlot	2nd	14.4%	26.8%	18.0%	X	X	X	X		4	**21.0%**	0.6%
			cab-sauv.	2nd	0.8%	3.5%	0.6%	X		X			2		< 0.1%
2006	-/+	Cadaujac	merlot	2nd	0.0%	0.6%	1.40%	X		X			2		0.1%
			cab-sauv.	2nd	0.0%	0.0%	0.90%	X		X			2		< 0.1%
		Latresne	merlot	2nd	2.6%	12.4%	7.30%	X	X	X	X		4		0.4%
			cab-sauv.	2nd	0.0%	2.5%	0.60%	X		X			2		< 0.1%
2007	-	Cadaujac	merlot	2nd	0.0%	0.0%	0.0%	X		X			2		0%
			cab-sauv.	2nd	0.0%	0.0%	0.0%	X		X			2		0%
		Latresne	merlot	2nd	2.9%	7.4%	7.9%	X		X	X		3		2.7%
			cab-sauv.	2nd	1.4%	1.4%	1.8%	X		X			2		0.2%
		Landerrouat	merlot	2nd	0.0%	0.0%	0.0%	X		X			2	0.0%	0.0%
		Mauriac	merlot	2nd	0.0%	0.0%	0.0%	X		X			2	0.0%	0.0%
		Monsegur	cab-franc	2nd	0.0%	0.0%	0.0%	X		X			2	0.0%	0.0%



Years: **8** for 2008, **9** for 2009
Regions: **W** for West vineyards (Bordeaux, Cognac) [blue bars]
E for East (Beaujolais, Mâconnais, Jura, Champagne) [green bars]
S for South (Languedoc-Roussillon, Côtes du Rhône) [orange bars]
Cultivars: **Ca** Carignan, **CF** Cabernet-franc, **Ch** Chardonnay, **CS** Cabernet-sauv., **Gr** Grenache, **Me** Merlot, **Mo** Mourvèdre, **Pn** Pinot noir, **Sy** Syrah, **Tr** Trousseau, **Ug** Ugni blanc

Figure 1: Number of treatments applied against powdery mildew and severity on bunches assessed at harvest in parcels within national network in 2008 and 2009.

Experiment within national network (2008-2009)

In 2008 and 2009 powdery mildew epidemics were very low in French vineyards, except for South regions mainly on Carignan cultivar. According to the disease indicators levels, only the 2 systematic treatments T1 and T3 were applied in 41 out of the 59 parcels (around 70%) experimented within the national network (Figure 1). This was the case in almost all the West and East regions sites, leading perfect level of grapes protection whatever the cultivar. However, two parcels received 3 treatments; leading to either 2.5% of disease severity on bunches at harvest (West) or no diseased berries (East), respectively. One parcel received 4 treatments without any damage (West). In the South region, higher disease levels at harvest were observed: around 30% of the parcels were sprayed 2 times (T1-T3), and 20% received 3 treatments. Half of these parcels, showed more than 1% disease severity on bunches at harvest, on Carignan, Chardonnay and Merlot cultivars. The other 50% of the parcels received 4 or 5 treatments leading good control of grapes infections except for 2 cases with 5% disease severity at harvest on Carignan and Merlot. On the very susceptible Carignan cultivar, full program with 5 treatments, at least 4, was necessary to reach successful disease control.

In all the situations, disease levels at harvest were considered acceptable by the growers in accordance with their objectives of production in quantity and quality.

In near 40 among the 59 sites experimented within the network, it was possible to compare the decision rule strategy with the vine growers practices both applied in

the same parcel. Disease control level was often better with grower program (not presented), but always with a higher number of sprays (Figure 2). According to the rule strategy, 2 to 5 fungicide applications are allowed in the season. The minimal program (T1-T3) was applied in 73% of the parcels. Under the same conditions, the growers applied 3 to 8 treatments, and more than 4 treatments in 75% of cases.

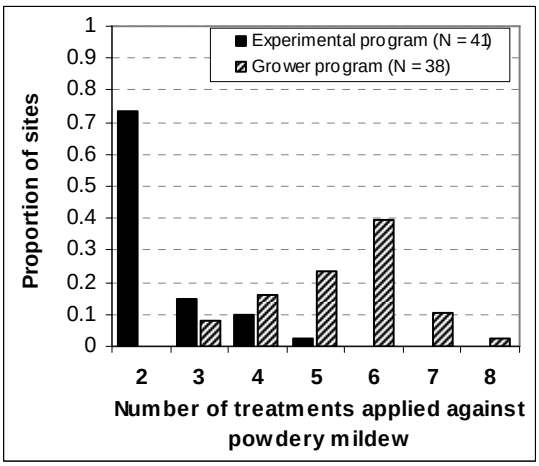


Figure 2: Distribution of vineyard parcels in number of treatments applied against powdery mildew within national network using the decisional rule (41 parcels) and vine growers practices (38 answers) during 2008 and 2009 seasons.

Conclusion

Early disease symptoms assessment can be useful as indicators to characterize powdery mildew epidemic level in grapevine parcels

Based on early disease symptoms assessment, the decision rule applied allows to reduce from 35 to 65% of treatments.

This confirm that the timing of treatments as define by the decision rule is appropriate.

Perspectives

The pm decision rule is now incorporated into a decisional process for the management of the combined protection against downy mildew and powdery mildew, called "GrapeMildews" ("Mildium" in French language), and evaluated within the national network expanded to 56 plots in 2010.

The current project, supported by the French Ministry of Agriculture, is a multidisciplinary program that combines the skills of pathologists, agronomists, economists and sociologists, within increasing national network in partnership with vinegrowers, extension services and schools of viticulture. In this program, the GrapeMildews process is taken as reference to study the technical feasibility and economic viability of input reduction and the acceptability of growers to change practices in viticulture.

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Toward establishing low input regimes in Australian viticulture 3: Use of 'epi-season' and 'lag phase control' in applying epidemiological knowledge of grapevine powdery mildew, to reduce the number of sprays and inoculum reservoirs for long-term control

P.A. Magarey[a],M.M. Moyer[b]

[a]Magarey Plant Pathology, PO Box 220, Loxton, South Australia 5333, email: pmagarey@riverland.net.au;
[b]NYSAES, Cornell University, Geneva, New York, USA, 14456.

There is simplicity about the epidemiology of powdery mildew. This is especially so in Australia:

- The only effective host of *E. necator* is *Vitis vinifera* vines within the vineyard;
- Most early-season inoculum travels <100 m from its point of origin until ~Day 80 from budburst (Magarey *et al.* 2010b);
- There are few home-grown or poorly sprayed *V. vinifera* to provide an external source of early-season inoculum within 200-300 m of most vineyards;
- In most regions, the disease progress is consistent, independent of leaf wetness and temperature because canopy microclimate is frequently optimum for growth (Messelink *et al.* 2000);
- The powdery mildew fungus grows externally on vine foliage, exposed and vulnerable to UV light and fungicide sprays; but albeit,
- In a typical annual management cycle, the disease begins early-season despite fungicide applications. It multiplies at steady rates, unseen by most. Bombarded with more fungicides and eventually controlled, crop loss is minimal but a legacy of a renewed supply of over-wintering inoculum remains.

The outcomes are significant economic cost, mostly from the intensive fungicide applications, and a high level of uncertainty as to whether crops at harvest will be sufficiently free of powdery mildew for acceptance by wineries.

A recent review of vineyard spraying practices in the Riverland (near Loxton, South Australia) showed that an unacceptably high percentage (~1-2%) of growers lost their crop when wineries, holding to low disease acceptance thresholds, rejected their grapes at harvest (Magarey *et al*, 2010a). Conversely, many growers controlled the disease well with an average of 6-7 fungicide applications/season. Some applied 12-13 sprays at high cost both economically and in levels of vineyard inputs. Many applications were inefficient, poorly timed or unnecessary. Interestingly, some growers achieved successful powdery mildew control with just 3-4 sprays.

A recent, innovative, sector-agreement between the South Australian wine industry and the State government aims to improve the 'clean green image' of South Australian wines in international markets. The agreement pre-supposed the practicality of reducing vineyard inputs so that strict targets were set in terms of greenhouse gas emissions and carbon credits. In practice, the cost of irrigating and harvest are relatively fixed and unavoidable but the cost of fungicide and fuel to manage powdery mildew is the highest variable-cost in South Australian viticultural production. In consequence, the Riverland grape industry proposed a spray regime of 3-4 fungicide applications/season as a preliminary benchmark for effective control of the disease.

A better understanding of the epidemiology of powdery mildew by grape growers has potential to facilitate progress toward this benchmark, *viz.* to improve the precision of spray timing, leading to more efficient control with fewer fungicide applications (Magarey *et al.* 2010a). When discussing the epidemiology of powdery mildew disease with grapegrowers and technical advisers in Australian viticulture, the simplicities of the epidemiology and their implications often seem lost and so, remain poorly understood.

Extension approach. One source of confusion (loss of simplicity) for growers appears to be the anomaly in plant pathological jargon when talking about the pathogen and its disease cycle *i.e.* events are presented in a *circle*, as compared to the growth of the grapevine expressed over the duration of a growing season(s), *i.e.* presented over a time *line*. In addressing this, we noted that figuratively, a life cycle can be 'cut open and stretched out' to be illustrated linearly over consecutive growing seasons.
In attempt to rectify this situation, we discuss here a way to present the key concepts in powdery mildew epidemiology to promote understanding of the disease by growers and their technical advisers. We offer these for review prior to fuller application within the Australian grape industry, as part of the process to optimise spray timing and frequency, and to improve the long-term control of powdery mildew with minimum vineyard inputs.

Epi-Seasons. Figure 1 presents the duration of an epidemic of powdery mildew (*i.e.* the 'season of epidemic', or, the 'epi-season') over a rolling window of two growing seasons. This approach highlights when the formation of primary inoculum occurs in relation to the progress of the epidemic. In the case of flag shoot inoculum which is the prime source of inoculum in inland regions of Australian viticulture, most buds that carry inoculum from the first season (Season 1) to supply conidia for the second growing season (Season 2), are infected early in Season 1 (Emmett *et al,* 2006). Notwithstanding, it is recognised that some infected buds may lie dormant for several seasons.

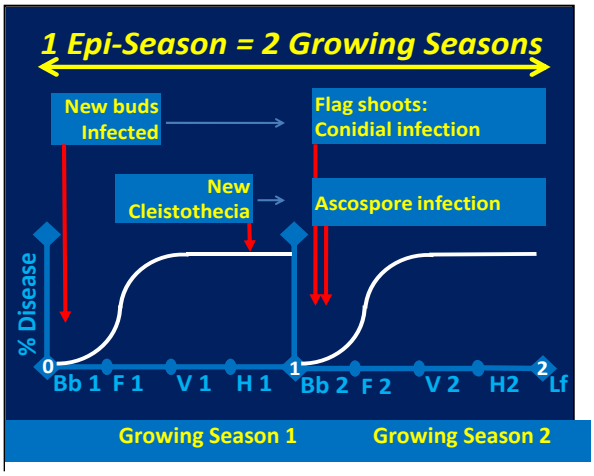

Figure 1. Schematic illustration of an epi-season (season of an epidemic) which, for grapevine powdery mildew, encompasses two growing seasons. The factors that influence the development of an epidemic are also presented *viz.* the number of infected buds and cleistothecia; where Bb=budburst, F=flowering, V=veraison, H=harvest and Lf=leaf fall, in Seasons 1 and 2 respectively. The white graph lines are disease progress curves in an unsprayed vineyard. In Australia, high levels of overwintering inoculum from Season 1 result in increased management difficulties in Season 2.

In contrast, the development of primary inoculum from cleistothecia occurs late in Season 1 and these fruiting bodies are discharged (producing ascosporic inoculum) sometime either prior to or early in Season 2 and they do not carry over to a third growing season.

Thus, since the inoculum from a neighbouring vineyard appears of little relevance in initiating epidemics (Magarey *et al*, 2010a), the progress of the epidemic within the vineyard in Season 1 determines the potential level of primary inoculum available to trigger infection in Season 2. At least in most Australian vineyards, this carry-over of inoculum from within a vineyard is the main factor influencing initial levels of disease in Season 2 and thus the level of difficulty faced by a grower to achieve good control of powdery mildew. We have found in preliminary presentations of the epi-season concept that growers have been encouraged in their thinking of how their management of disease at various times in the current growing season affects the levels of inoculum that carries-over to the next.

Lag phase control. In an unsprayed, inland Australian vineyard, an epidemic of powdery mildew progresses under more or less continuously optimal conditions, represented in graphical form by a typical log curve with uniform rate of increase (Emmett *et al,* 2006). In consequence, the best defence against fruit infection begins with preventing leaf infection and this is best achieved early in the life of the epidemic.

Figure 2 shows graphs of the increase in disease incidence and severity. In inland Australian vineyards, disease incidence regularly increases significantly at around Day 40 from budburst, and severity, from Day 80.

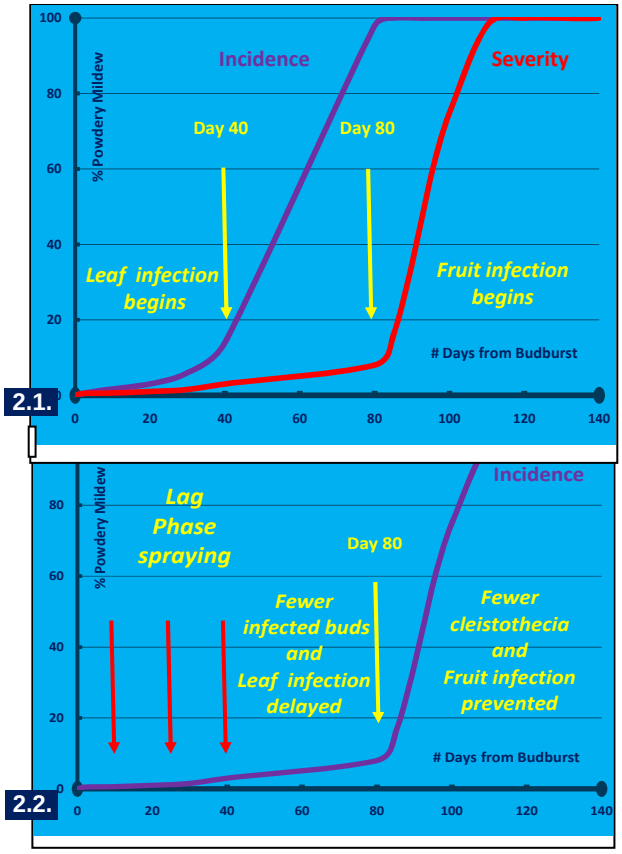

Figure 2.1. Graph illustrating the timing of the increase in incidence and severity of grapevine powdery mildew in an unsprayed, inland Australian vineyard (data from Emmett *et al,* 2006). Disease incidence increases significantly at around Day 40 from budburst and severity increases at about Day 80. Figure 2.2. 'Lag phase control' strategies apply fungicides while initial inoculum levels are low and more manageable in Season 1 at critical times and sufficiently early in the epi-season to: 1) increase the length of the lag phase and 2) prevent the development of over-wintering inoculum that increases disease potential in Season 2 (Emmett *et al* 2006).

As a result, optimum control strategies aim to reduce disease incidence before levels increase rapidly *i.e.* in the lag phase before Day 40.

Superimposing the two concepts *viz.* 1) control disease in the lag-phase, and 2) manage disease at critical points in the epi-season, helps demonstrate that excellent control of disease in Season 1 is achievable with:

- Fewer fungicide applications required later that season because sprays are applied when inoculum loads are low (before the log phase of increase); and as a result,
- Fewer sprays in total for Season 1;
- Lesser potential for rejection of crops in Season 1;
- A reduced carry-over of inoculum to Season 2 (*i.e.* fewer infected buds and fewer cleistothecia) and thus less disease potential for that season (Figure 3); and as a result,
- Fewer sprays to achieve early season control in Season 2.

Fungicide applications during times of low spore load reduce the risk of pathogen tolerance to susceptible fungicides. The application of this simplified understanding of disease epidemiology has shown potential to achieve the above objective and thus, to reduce or eliminate local sources of inoculum (Magarey, 1998).

Presentation of the epi-season and lag phase control concepts to grapegrowers in the Riverland region and beyond have, to date, been well received. The most common responses cited have been: a better understanding of the role of initial inoculum in the development of epidemics; appreciation of a simplified approach to the epidemiology of disease; encouragement to improve the efficiency of controls with fewer sprays; and interest in the possibility of achieving a long-term reduction in inoculum reservoirs by effective control over successive seasons.

The possibility of attaining the new industry benchmark of 3-4 fungicide applications/season to successfully control powdery mildew and enhance the clean green image of South Australian wines looks promising. This could assist in reducing vineyard inputs, optimising the number of sprays and minimising the fuel consumed to control the disease with success. Since viticulture in the Riverland, South Australia, is similar to that in Sunraysia near Mildura, VIC, and Riverina, near Griffith, NSW, this approach has potential for acceptance across much of Australian viticulture.

Other pathosystems. It is easy to foresee how simplifying disease concepts to more closely align host development and long term disease management can be applied across a broad array of pathosystems. One such example is with grapevine downy mildew. For this pathosystem, the episeason would extend over several growing seasons due to the longevity of oospores in the soil. This would really highlight the importance of in-season disease management for long-term epidemic control.

The epi-season concept also has potential to include other aspects of the culture of the grapevine as these influence the expression of disease *e.g.* irrigation and nutrition practices and their influence on attempts by growers to manage the vineyard dynamic that leads to disease expression.

Summary

In inland Australian viticulture, powdery mildew incurs significant cost in terms of frequency of fungicides applied and high levels of vineyard inputs such as fuel usage. This occurs regularly despite inherent simplicity in disease epidemiology and attempts to convey epidemiological knowledge to grape growers.

Simplifying communication of epidemiological concepts to growers and technical advisers by more closely aligning pathogen biology to host biology has potential to improve the efficiency and effectiveness of controls applied for powdery mildew. The application of simple concepts of disease epidemiology is proposed to assist growers understand critical aspects of disease management with potential to improve the effectiveness of controls and reduce local inoculum reservoirs. The possibility of reducing vineyard inputs to achieve grape industry targets of improved control of disease with fewer spray applications looks promising.

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Effect of sunlight, specifically ultraviolet radiation and increases in surface temperature, on grapevine powdery mildew development

C. N. Austin[a], W. F. Wilcox[a]

[a]NYSAES – Cornell University, 630 West North Street, Geneva, NY 14456

A report on the dramatic effect that ultraviolet (UV) radiation and surface temperature have on powdery mildew (PM) development, and how optimizing sunlight exposure through appropriate canopy management practices, can reducing PM in the vineyard. Controlling powdery mildew, caused by the fungus *Erysiphe necator*, is an essential component of any vineyard management program. Most *Vitis vinifera* cultivars are highly susceptible to this disease and some, like Chardonnay, are extremely susceptible. Furthermore the disease is active across a wide range of weather conditions, and rainfall is not essential for disease development.

Numerous observations have associated an increase in grapevine powdery mildew with shade. Sources of shade can vary within a vineyard from; vine training systems, pruning level, row orientation, geographic location, fruit-zone leaf removal, adjacent hedgerow shading, time of year, cloud cover, or self-shading (Reynolds and Vanden Heuvel, 2009, Dokoozlian and Kliewer, 1995, Calonnec et al. 2009). Basal leaf removal around fruit increasing sunlight exposure has been associated with creating a microclimate unfavorable for PM (Chellemi and Marois 1992). Vigorous vines have been shown to be a significant source of PM inoculum (Calonnec, 2009) for a vineyard. Training systems and row spacing influence sunlight distribution within a vineyard and affect PM disease severity (Zahavi *et al.,* 2001), independent of ambient temperature or relative humidity. Vines grown under sunlight which has had its ultraviolet (UV) radiation removed have significantly more PM disease than vines exposed to UV radiation (Keller 2003). Willocquet *et al*. (1996) demonstrated that *E. necator* conidia on leaf discs exposed to UV-B led to reduced conidial germination and mycelial growth.

Inherent with increases in direct sunlight exposure are increases in ultraviolet radiation. This component of sunlight (in particular UV-B) has been studied for its numerous biological effects. UV-B has been shown to alter fungal populations on plant leaves (Moody *et al*. 2001); change grapevine physiology (Kolb *et al*., 2001), and generally increase fungal mortality (Rotem *et al.*, 1985). Recent studies also stress further importance in studying the effects of UV-B radiation in agricultural systems in relation to current decreases in stratospheric filtering of UV-B radiation (Björn, 2007). Although the response to UV-B radiation is species/pathogen specific (Roberts and Paul, 2006) most instances are characterized by a negative correlation with pathogen growth and UV-B exposure.

The second component associated with sunlight that inhibits PM is the leaf or berry surface temperature derived from heat transferred to vine surfaces intercepting sunlight. Surface temperatures on exposed leaves and fruit can be 5-15°C higher than the surrounding air temperature. Powdery mildew grows fastest at 25-28°C (Delp, 1954), ceases to grow at temperatures above 32°C, and starts to die if temperatures remain over 35°C for a sufficient length of time. Our hypothesis was that both components of sunlight influence PM development in the vineyard, and potentially interact. Secondarily, we aimed to show that viticultural practices influencing sunlight distribution in the canopy will alter PM severity.

Results

In order to investigate, and separate, the two inhibiting components of sunlight under vineyard conditions, Plexiglas was suspended over vines in both Geneva, NY (interspecific hybrid Chancellor and Chardonnay). Vines underneath the Plexiglas received the longer wavelengths of sunlight that elevate surface temperatures, yet were protected from UV radiation. For Chancellor vines, in addition to Plexiglas, shade cloths were suspended over separate vines. Underneath the shade cloth, vines were shielded from damaging UV radiation and also from the solar components that heat up leaves and fruit. Clusters inoculated with a suspension of PM conidia (100K/ml) at 75% capfall and fungal diseases on these vines were managed with minimal applications of non-volatile fungicides, during which test clusters were protected with individual plastic bags. As shown in Figure 1, disease development was least in the exposed fruit and reducing UV radiation increased disease severity on fruit 2 to 5 times, for both varieties. Avoiding the increase in surface temperature alone also increased disease severity in one year for Chancellor vines. As surface temperature is an indirect effect, this may rely more on other variable vintage conditions, such as rainfall, vine water status, and ambient temperatures. Poor fruit set in the Chancellor vineyard in 2007 prevented disease severity ratings to be collected.

Growth chambers maintained at 20°, 25° or 30°C were used to examine the effects of UV and surface temperature separately, and to determine any interaction. Detached chardonnay leaves were dry inoculated by direct contact with 14-day old PM colonies and placed into the chambers.

For each temperature, leaves were exposed to a 6-hour dose of 3.0 W/m² from a narrowband UV-B bulb for each of 0, 1, 2, or 4 days for a total radiation dosage of 0, 21.6, 43.2 or 86.4 kJ/m^2, respectively.

The increased doses of UV-B radiation inhibited germination and growth of PM. In addition, the inhibitory effect of UV-B radiation on latent period was exacerbated at higher temperatures. As shown in Figure 2A, different UV-B doses had a similar slowing effect on latent period at both 20 and 25°C but at 30°C, all UV-B exposures increased the latent period, with four UV-B doses extending latent period from 7 to 15 days. UV-B radiation has a stronger retarding effect on latent periods at warmer temperatures. Increasing doses of UV-B inhibited hyphal growth in a negative linear relationship (Figure 2B).

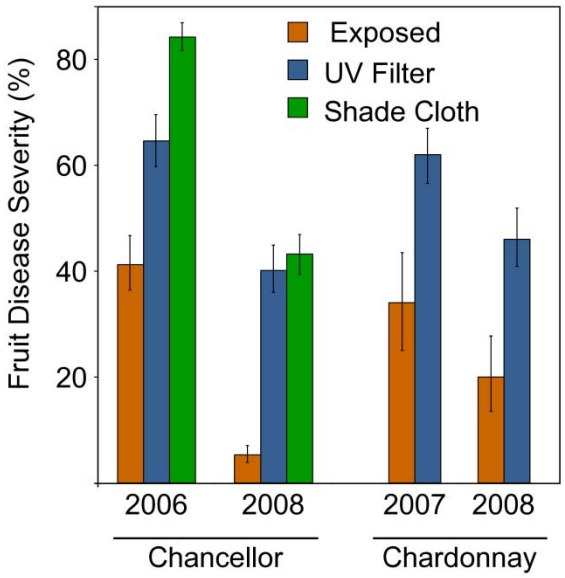

Figure 1: Percent disease severity on Chancellor and Chardonnay vines receiving: full solar radiation (Exposed), sunlight from which 95% of the UV radiation had been filtered (UV Filter), or sunlight reduced to 20% of ambient via neutral density shade cloths suspended over vines (Shade Cloth). Clusters were inoculated with a suspension of PM conidia (100k/ml) at 75% capfall. Vineyards were located in Geneva, NY.

There was no significant interaction between UV-B dose and temperature on hyphal growth.

In a young Chardonnay vineyard in Geneva, NY, two forms of canopy manipulation, training system and basal leaf removal provided variable light exposure to the fruiting zone. For the training system Vertical Shoot Positioning (VSP) and Umbrella-Kniffen (UK), were compared with the latter providing more shoots per linear meter of row and, thus, at risk for increased canopy shading in the fruit zone. Within each training system, leaves were removed at two dates: 2 weeks post-bloom (fruit set) and 5 weeks post-bloom, and for each date either two leaves were removed above and below the cluster (heavy), one leaf removed above and below each cluster (light), or not at all (control). Clusters were inoculated with a suspension of powdery mildew conidia (100K/ml) at 75% capfall and fungal diseases other than PM were managed with minimal applications of mancozeb and Stylet-Oil [a paraffinic oil], during which test clusters were enclosed in individual plastic bags. As shown in Figure 3, (i) PM severity was lower in the VSP compared to the UK training system, regardless of leaf removal. Using multiple regression analysis, we determined removal of leaves at fruit-set, within each training system, significantly reduced the amount of PM on berries ($p<0.05$), whereas removal 5 weeks after bloom had no significant effect, nor did the number of leaves removed. The benefits of early leaf removal are likely explained by the temporary high susceptibility of fruit to powdery mildew infection from the early bloom through fruit set period. Without the use of fungicides, a VSP training system and basal leaf removal at fruit set, reduced fruit disease severity by 35% relative to UK-trained vines with no leaf removal.

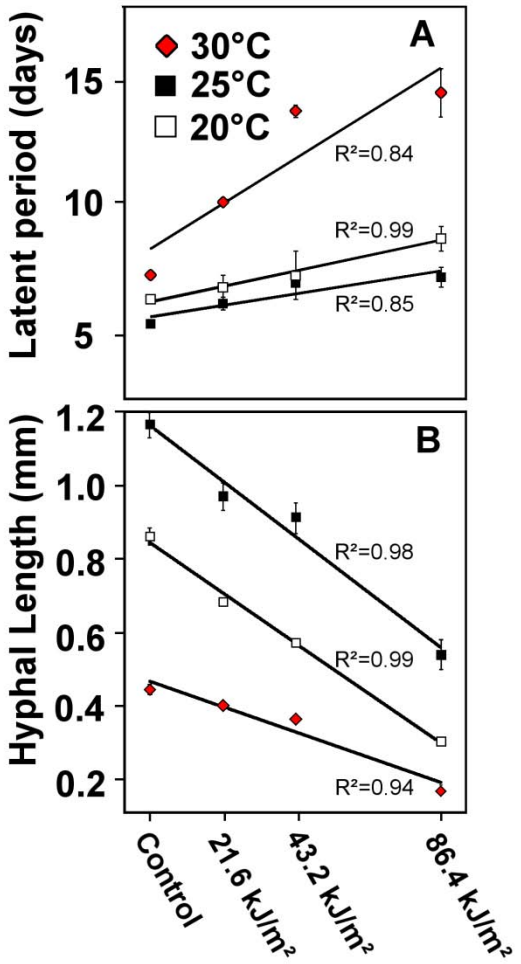

Figure 2: The effects of UV-B, temperature, and their interaction on PM development. Inoculated Chardonnay leaves were maintained at either 20, 25, or 30°C and, beginning immediately after inoculation, received 6-hour doses of 3.0 W/m² UV-B radiation for each of either 0, 1, 2, or 4 days for a total radiation dosage of 0, 21.6, 43.2 or 86.4 kJ/m^2, respectively, **A**. Effects of temperature and UV dosage on that latent period of PM. **B**. Mean maximum hyphal length for each temperature x UV-B combination for conidia. For each of three replicate leaves the longest hyphae from 10 conidia were measured 96h post inoculation.

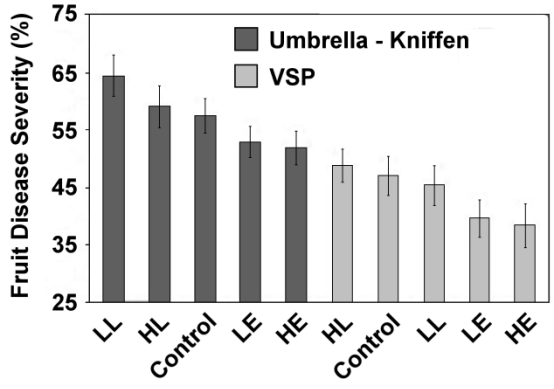

Figure 3: Powdery mildew severity on Chardonnay fruit subjected to five different leaf-removal treatments in each of two vine-training systems. Clusters were inoculated at bloom with a suspension of PM conidia (100K/ml) and disease severity was visually assessed on a 0-100% scale. Leaf-removal code: **First letter** is leaf removal severity, H = heavy, L = light (either two leaves or one leaf above and below each cluster, respectively); **Second letter** is leaf removal timing, E = early, L = late (2 and 5 week post-bloom, respectively).

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Toward establishing low input regimes in Australian viticulture 1: A review of powdery mildew control in vineyards of the Riverland, South Australia.

P.A. Magarey[a], R.W. Emmett[b], T. Smythe[c], J.R. Dixon[d], M.M. Moyer[e], A. Pietsch[f]

[a] Magarey Plant Pathology, PO Box 220, Loxton, South Australia 5333 (formerly South Australian Research and Development Institute, Loxton, SA 5333), email: pmagarey@riverland.net.au; [b] Dept. Primary Industries, Mildura, Vic 3502; [c] Riverland Wine Industry Development Council, Berri, SA 5343; [d] South Australian Murray-Darling Basin Resource Information Centre, Berri SA 5343; [e] NYSAES, Cornell University, Geneva, New York, USA, 14456; [f] CCW Co-op. Ltd, Berri, SA, 5343.

Many grape growers in the Riverland district (near Loxton), South Australia, spray frequently to control powdery mildew (*Erysiphe necator)*. Most achieve good control but at a substantial cost. A single, region-wide application of sulphur, for instance, costs ~AUD$970,000, while the total cost of sprays each season is ~AUD$4-8 million. Additional industry impact occurs through the carbon costs cited against the high fuel consumption with frequent spraying and associated increased pressure upon the grape industry to reduce emission of greenhouse gasses. The Riverland grape industry initiated this research to explore a more targeted approach to powdery mildew management and the potential for localised eradication of the disease.

To accredit grapes supplied to wineries at harvest, growers are required to maintain spray diaries with details of fungicides applied, timings, rates and spray volumes. These records offered potential to evaluate vineyard spray programs, increase financial and environmental efficiencies and rationalise spraying practices for an industry under increasing demand for low (critical) input viticulture and sustainable management decisions.

The aim of the project was to 1) review vineyard spray diaries and associated records of mildew levels in the vineyard; 2) investigate control successes and failures; and 3) assess the potential for a lower input spray regime. Matters of particular interest were: to develop an understanding of what drives good powdery mildew control; to determine what causes the failures; and to determine the scope for change in disease control and environmental and financial performance.

Methods

Data from paper-based spray diaries supplied by local wineries from the 2006/07 season were entered onto the computer and a 'vineyard spray program evaluator' (based on MS Access®) was devised and deployed to rapidly assess the accuracy and potential effectiveness of each spray record in each diary. An assessment of spray programs for each vineyard patch by the evaluator was compared with a mildew score assigned by winery assessors prior to harvest for that patch. This allowed review of the diaries as an initial sample of Riverland (and Australian) vineyards, to estimate the number and effectiveness of sprays for powdery mildew.

Spray diaries from 2006/07 were reviewed for 1,206 *Vitis vinifera* patches of *cv.* Chardonnay, 1,450 Shiraz and 13 Verdelho, while spray diaries from 160 *cv.* Chardonnay growers were assessed in detail.

Results

Overview. The analyses showed that powdery mildew was well controlled in 73% of Chardonnay patches; a large majority of growers thus achieved excellent control of the disease. Only 6% of growers failed to control the disease, having their fruit rejected by the winery at harvest. Further analyses of the spray programs from 137 vineyard patches showed that individual spray programs were extremely variable. Some growers sprayed for powdery mildew only twice in the season while others sprayed 13 times. The average was 6-7 applications/season. Of the total number of growers, 25% sprayed less than six times. However, although a majority (75% of the surveyed growers) sprayed less than eight times, a reasonable proportion (25%) applied more than eight sprays.

The variability in these numbers indicated a lack of recognition of optimum timing of spray applications. The outcome was unnecessary expenditure on fungicides, petrol, labour and water. The extra spraying may have been the result of poor spray timing leading to additional sprays in attempts to 'catch up'. Further education was needed to help growers decide when to spray and how many sprays to apply for reliable control.

Spray timing scores. The need for well-timed sprays was correctly perceived by many growers but, for a number of growers, this appeared to translate to 'applying more just to be sure'. A review of the spray records indicated that this approach was followed frequently, without achieving improved control, efficiency or economy (Figure 1).

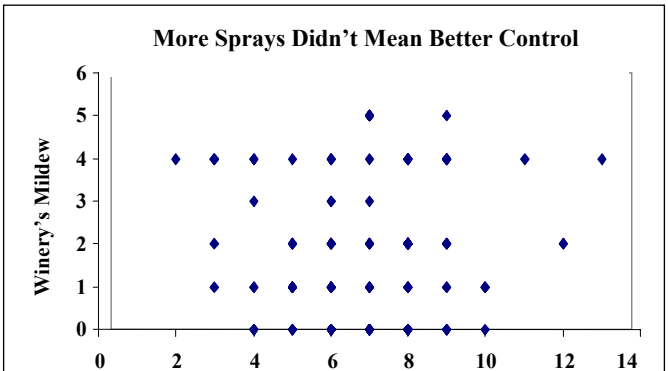

Figure 1: Total number sprays/season for powdery mildew in the Riverland, near Loxton, South Australia, 2006/07, indicated that better control of powdery was not achieved when more sprays were applied.

There was no connection between the total number of sprays and the disease ratings in patches. Some vineyards with low disease scores received the minimum number of sprays (2/season), while others received the maximum number (13/season) (Figure 1). Hence, more sprays did not give better control. Instead, it appears that more appropriate timing of sprays was needed for better control.

A closer look at the spray diary data showed that Chardonnay patches with excellent disease control *i.e.* with a very low mildew disease rating, received as few as four and as many as 10 sprays. This illustrated that the best control for powdery mildew could be achieved with as few as four sprays. In more tolerant varieties or where inoculum loads are reduced, even fewer sprays may be required.

Rating diaries for spray timing. To check this in more detail, a Spray Timing Score was developed within the spray diary evaluator to rate the date of each spray within each diary in relation to a supposed ideal timing for good disease control. A scale of 1-20 was established, based on knowledge of powdery mildew epidemiology and the susceptibility of vines to powdery mildew at the different stages of the season (Magarey and Emmett, *unpublished data.)*. From this, a theoretical 'best timed' spray strategy was developed. A patch with a score of less than 6 was considered to have very good timing, and a patch with 1 to 3, excellent timing. Patches with very poorly timed sprays, often missing key target windows for powdery mildew development, was given a score of 10 or more. The analyses of 'Spray Timing Score' in Riverland spray diaries showed a 'two-peak' distribution (Figure 2).

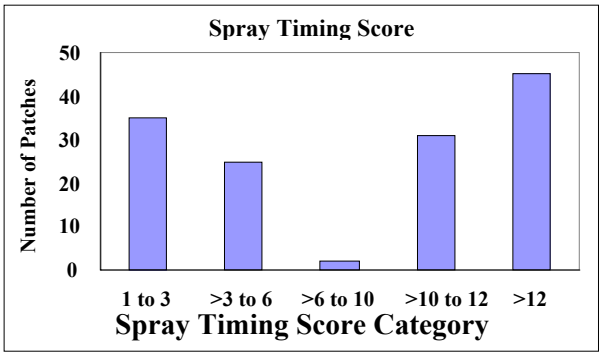

Figure 2: Distribution of Riverland, South Australia, vineyard patches in 'Spray Timing Score' categories in relation to a theoretical optimum spray schedule – the system ranked scores from 1 to 20.

Most patches were ranked in one of two extremes. About 45% of growers performed very well in relation to the theoretical optimum spray timings (with Spray Timing Scores ≤ 6) while nearly 55% of the diaries had very poor spray timing (with scores ≥ 10). This indicated that while a large proportion of growers were, we suppose, well educated and vigilant with their spray timing, there was a slightly larger proportion who appeared to lack either the knowledge of powdery mildew disease or the confidence to back their judgement on that knowledge. Either way, the second group timed sprays poorly and failed to control the disease efficiently. Patches with a mildew Disease Rating of zero (excellent control) achieved this with both a (few) well-timed sprays, or with (many) poorly timed applications. And patches with a high Disease Rating were sometimes sprayed with well-timed applications, without apparent effect.

Spray Treatment and Technique Scores. Analysis of the spray diaries also included a score for: a) 'spray treatment', judged on the appropriate choice of fungicide and rates of application at a particular time, and b) 'technique', judged on use of an appropriate water volume and according to the number of other products tank-mixed with the selected powdery mildew fungicide. All growers, in all patches and at all sprays, received a perfect score for treatment! The correct choice of fungicide type was universal within our sample set of diaries. Overall, most growers also received good scores for technique, however, a few (8 growers) tank-mixed more than six products.

Eichhorn-Lorenz (E-L) growth stage. Various educational issues were also highlighted in this study. One was the inability of many growers to use the E-L system to correctly identify vine growth stages. Many growers had a limited knowledge of this system at stages apart from the flowering stage. If the E-L system is still to be used in spray diaries, a better understanding of it is needed.

Spatial analyses of spray diary data. A geographical representation of the spray diary data showed a grouping of vineyards with poor control in several localities. The cause of these anomalies was not known but may have related to a locally high level of disease that made control difficult, or may be, some sociological/cultural factor relating to the approach used by local growers attempting to control disease, or perhaps some local meso-climate influence. A more likely explanation is that some of these vineyards may have the same owners using the same inefficient strategies for disease control. The actual cause needs elucidation.

Influence of Irrigation Practices on Severity of Powdery Mildew. Of significant note, is that higher incidences of powdery mildew occurred in patches with under-vine sprinkler irrigation in comparison with drip irrigation (Table 1). This was observed in the detailed assessment of records from both Chardonnay and Shiraz growers.

Table 1. Disease Rating associated with irrigation practices in a sample of Chardonnay vineyards, Riverland, South Australia. 2006/07 (n=137).

Powdery Mildew Disease Rating	% Patches with Drip Irrigation /Total # (n=23)	% Patches with Under Vine Sprinklers /Total # (n=70)	% Patche O/Head Sprinklers Total # (n=36)
0	35%	38%	25%
1-3	12%	49%	31%
4-5	3%	**73%**	17%

Conclusions

Improved spray programs with fewer sprays/season have potential for better control of powdery mildew in the Riverland region of South Australia. Since this region is representative of much of inland Australian viticulture, the implications potentially have a much wider application.

- As few as 2-4 sprays/season may be adequate for good control of powdery mildew, allowing environmentally cleaner spray programs and lower cost of disease control.
- A better understanding by growers of the epidemiology of powdery mildew development in the Riverland may improve resolution in the timing of at least some sprays.
- The 'spray evaluator' appeared to function well for Riverland diary records but existing diaries do not record and therefore, do not permit assessment of, vineyard inoculum levels nor the effectiveness of spray coverage. This was a major limitation to the study and its capacity to better define the deficiencies in current spray strategies.
- Unsuccessful management of powdery within vineyards was restricted to a few growers in isolated patches. The high winery ratings they received for powdery mildew may be related to excessive inoculum loads, poor spray coverage or improper sprayer maintenance. It appeared that there is an education gap in disease management, especially with these

growers. The findings from recent research programs and the subsequent revision of spray strategies for *E. necator* (e.g. Emmett *et al*, 2006; *Magarey and Emmett, pers. comm.*) is yet to be fully conveyed to Riverland (and Australian) growers.

- Assessing spray diaries helped to identify areas for improvement in managing the disease in Riverland vineyards. Analysis of more diaries from additional years and cultivars has good prospects to develop a better profile on the activity of the Riverland industry in managing the disease at present and set a standard (bench-mark) for assessing the value of any future initiatives to improve spraying practices.
- An easily used, standardised, disease rating system has potential to improve the quality of disease assessments made by winery staff and assist the analysis of spray diaries by growers, vineyard monitors, managers and researchers.

Vineyard spray program evaluator. The 'evaluator' facilitated rapid assessment of the spray diaries. It processed and scored large numbers of digital spray records. Its key function was based on theoretical 'ideal' spray dates set according to our assessment of optimal spray timing established from field studies of disease epidemiology. The evaluator developed and used in this project could also be used to:

1) Rapidly review similar spray diary records for other seasons or other regions.
2) Develop industry bench-marks for fungicide use in the control of powdery mildew.
3) Evaluate planned spray schedules to review supposed effectiveness and weakness before sprays are applied, and so assist in determining which spray schedules are likely to be effective and which are not.

Thus, the evaluator has good potential to assist in reducing the number of sprays for improved control of mildew, reducing fuel and chemical use, lowering costs and carbon emissions in Australian vineyards.

The vineyard spray program evaluator has potential for growers, vineyard managers, consultants and others to assess their own records via an online module such as *CropWatchOnline.com*.

Riverland spray programs. Generally, the spray programs deployed by Riverland growers controlled powdery to levels with minimal impact on sale of the crop. There was however, a large range in the number of sprays applied to achieve this standard. Some growers achieved excellent control with as few as 2-4 sprays while others sprayed twelve or more times and achieved poor control. There was no correlation between the number of sprays and the level of control.

The assessment revealed there were two groups of growers: those who sprayed a lot and those who didn't. Either way, the correlation between timing and disease control was not strong, meaning that there was a lot of variation in what individual growers did in attempting to control the disease.

This review helped identify aspects of spray programs with potential for improvement in controlling powdery mildew. For instance, there was no correlation between the timing and treatment (i.e. the selection of registered fungicides) in control strategies. Means of achieving adequate spray coverage and an improved knowledge of the management of inoculum reservoirs appear to be issues that need addressing. This includes aspects such as where does the disease come from: flag shoots and/or cleistothecia? When does the over-wintering inoculum begin to spread? And, when is the best time to begin spray applications for long-term control of the disease?

Regarding the above, there appears to be scope for improvement within current industry practices, and opportunity to move to strategic spray programs with fewer well-timed sprays for better management of powdery mildew. More analysis of the spray schedules in this study and analysis of diaries from a second season would be useful to help define a valid bench-mark for change before introducing a structured approach to improved disease control across the region.

Not-withstanding this, it seems realistic for the Riverland grape industry to set a course to reduce the number and improve the effectiveness of sprays applied for powdery mildew control in the region. In the spray diaries we investigated, many growers achieved control with four sprays per season. It is recommended that this be set as a preliminary benchmark for the Riverland grape industry.

Since the climate and viticulture in the Riverland of South Australia, is similar to that in the Sunraysia near Mildura, Vic, and the Riverina, near Griffith, NSW, the above findings have potential for application across much of Australian viticulture.

Acknowledgement

The authors acknowledge the contribution and assistance of their late colleague, Peter Burne whose enthusiasm and assistance was highly valued, especially in the early stages of this project. He is sadly missed by the research team.

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Trials results of the 'Optidose' method using an adjustment of the pesticide dose for control of downy and powdery mildew

A. Davy[a], M. Raynal[a], M. Vergnes[a], S. Remenant[a], A. Michez[a], M. Claverie[a], S. Codis[a], FM. Bernard[a], L. Colombier[b], L. Davidou[b], M. Girard[b], L. Mornet[b], J-P. Perraud[b], C. Rives[b], D. Vergnes[b]

[a] IFV - 39 Rue Michel Montaigne - 33290 Blanquefort, France, [b] Chambres d'Agriculture Dordogne, Gironde, Charente-maritime, Charente, Landes, Lot et Garonne et Pyrénées Atlantiques, France

Under the climate in France, the use of pesticides is mostly essential for producing quality grapes. Blacklisted by consumers, their use is becoming increasingly contested and regulated by legislation. The challenge of the current project was to propose efficient strategies to reduce the quantity of pesticides used while maintaining an acceptable level of protection. The 'Optidose' concept proposes an adjustment in pesticides dose applied according to the situation (biomass, pest pressure ...) to minimize the use of chemical inputs.

The decision rules proposed

In France, the registered dose of fungicides remains in unit (L or kg) of product per unit of soil surface, whatever the stage of implementation or development of the vegetation. The registered dose is calculated to remain effective when conditions are favourable for disease development in fully developed vegetation, which is fortunately not always the case in practice.

Tests varying doses of fungicides have been undertaken by IFV Bordeaux since 1996. The decision rules used in the project OPTIDOSE proposes an adaptation of the fungicide dose applied to protect the vineyard, based on the plant surface, the diseases pressure and the development stage. A table (Figure 1) has been created to calculate and adjust the dose of fungicide for these different factors and for each treatment.

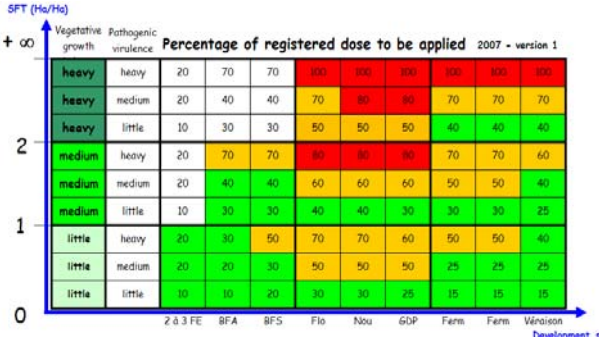


Figure 1: Table showing variations in recommended doses of fungicides as influenced by development stage, vegetative growth and pathogenic virulence - Version 2007

These decision rules are tested by IFV on micro plots trials but also on a collaborative experimental network with grape growers, developed in partnership with viticultural advisers of various vineyards departments (13, 16, 17, 24, 31, 33, 40, 41, 44, 47, 64, 73, 84). The trials and network enabled us to improve the decisions rules presented in Figure 1. The 2009 version is available at the site of the EPICURE IFV (http://www.vignevin-epicure.com).

Results of testing multi-adaptive doses of fungicides

In such trials, three forms were compared:
1: Untreated plot (TNT)
2: Optidose (doses adapted for downy and powdery mildew)
3: Reference (full dose or real dose used by the grower)

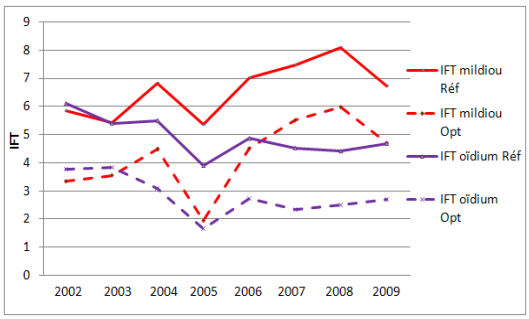


Figure 2: Yearly progression of the Treatments Indicator Frequency for downy and powdery mildew in varying dose trials from 2002 to 2009

The Indicator Treatment Frequency (ITF) is the number of full doses applied per hectare per year (2 treatments with half dose = 1 ITF). The average number of treatments is 8.2 for downy mildew and 5.9 for powdery mildew. The average percentage reduction of dose between optidose and the reference is 41% for downy mildew (-49% compared to registered full dose) and of 45% for powdery mildew (-52% compared to registered full dose). As shown in Figure 2, the number of treatments and the level of reduction of the dose varies depending on the climate and therefore pathogen pressure of each year.

Downy mildew results. The levels of pathogen pressure and damage observed in untreated controls are very dependent on the experimental sites and years, from 0 to 100% of the harvest. It is observed that the damage in the vineyards using Optidose are still slightly higher than the reference but they are acceptable in most cases.
On a limited number of tests, the "reference" condition itself does not have adequate protection. Depending on circumstances, there may be gaps in the protection, applied doses too low, bad quality of application or a combination of these factors. We notice that, when the reference itself doesn't give a good result, damages severity may increase on the Optidose condition, as far as to sometimes become unacceptable.

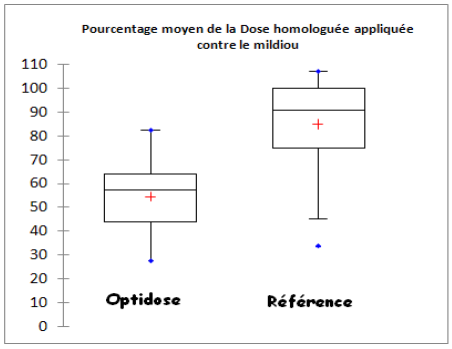


Figure 3: Average percentage of registered dose applied against downy mildew (trials from 2002- 2009).

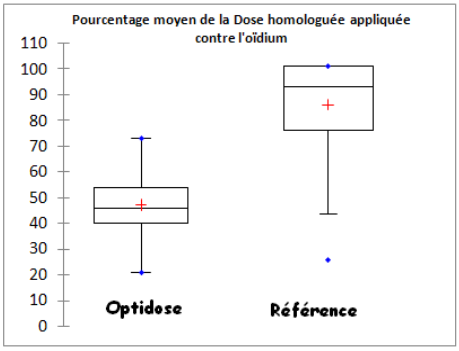
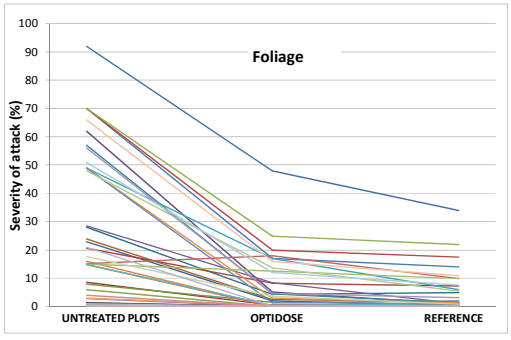


Figure 4: Average percentage of registered dose applied against powdery mildew (trials from 2002 to 2009).



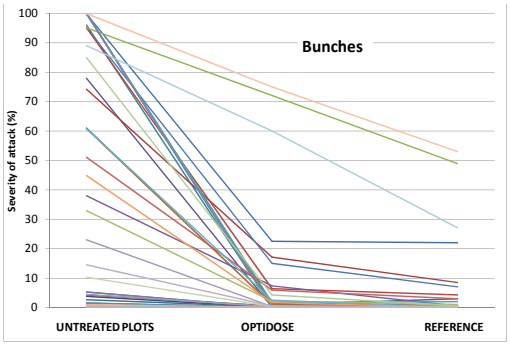
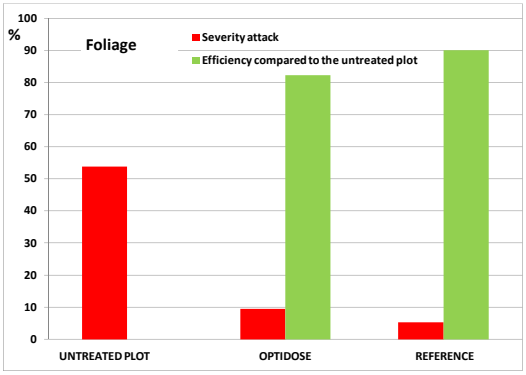


Figure 5: Severity of attack on foliage and grapes due to downy mildew at ripening stage.



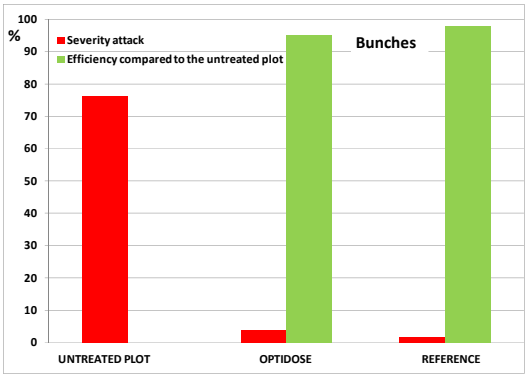
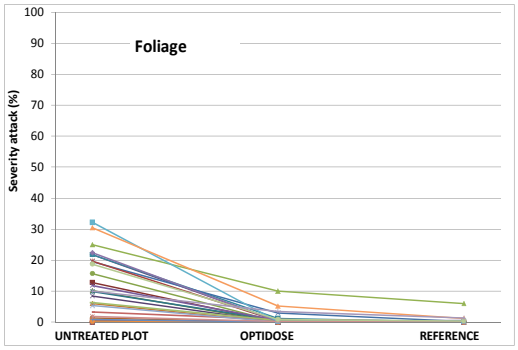


Figure 6: Severity of attack on foliage and grape bunches due to downy mildew at ripening stage.



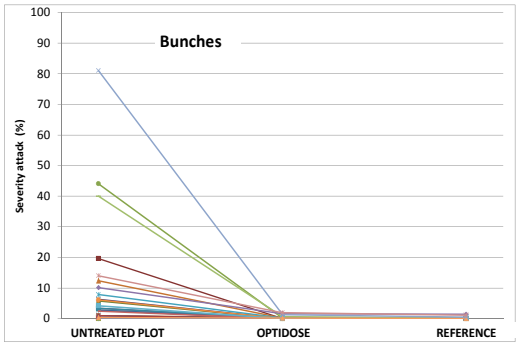


Figure 7: Severity of attack on foliage and grapes due to downy mildew at ripening stage.

When the protection generated by the registered dose gives good results, an overall decrease of 35% of chemical input doesn't have a significant impact on the efficacy of the protection. However, these averages may include some cases of extreme failures.

Powdery mildew results. The powdery mildew pressure was much less than that observed for downy mildew during these 2002-2009 trials. Logically, the damage caused by powdery mildew are much less important. Under these conditions of moderate pest pressure, the different strategies (reference and optidose options) showed good results even if, as for downy mildew, we always notice a bit more damage on vines managed by the optidose method .

Conclusion

Dose reduction when spraying vineyards for downy and powdery mildew is already largely empirically practiced by many growers. The goal of our project was to provide decision rules for spraying these diseases with optimum fungicide dose management in order to reduce the quantity of pesticide used without harming the efficacy of the protection of the vineyard.

In our 'Optidose' trials, an average dose reduction of about 40% frequently led to a low increase of symptoms. For these low or medium pressure years, the fungus attacks led to an insignificant additional destruction of about 1%. For years with high disease pressure, the damage increases were more important, around 10% on leaves and 4% on the grape harvest. However, these additional symptoms were also noted in the average reference practices of grape growers. The major risk in applications of the 'Optidose' option is that caused by a dose reduction in exceptional conditions, for which even the full dose sometimes gives unsatisfactory results; high levels of destruction can be seen in these cases.

The dose reduction we propose, leads to a reduction of safety margins. Under these conditions, the use of a well regulated and efficient vineyard sprayer becomes imperative.

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Session 3: Posters

Observation on grapevine downy mildew dynamics in two vineyards of the Venetian region

A. Zanzotto, M. Borgo

Centro di Ricerca per la Viticoltura, CRA-VIT, Viale XXVIII Aprile 26, 31053 Conegliano (TV), Italy

Downy mildew, caused by the oomycete *Plasmopara viticola* (Berk. *et* Curt.) Berlese e De Toni, is a source of major concern for the vinegrowers of the Venetian region.
Although some practical support to determine the putative infection dates are availabe, such as the so called "three 10s" rule (Baldacci *et al.*, 1947) and the percentage of theoretical incubation (Goidanich *et al.*, 1957), the real appearance of symptoms doesn't always respect the outputs from these tools. More recently, advances have been done on the way to predicting the infections of *P. viticola* and a series of previsional models have been elaborated: PRO, EPI, Plasmo, DMCast, UCSC (Caffi *et al.*, 2007). Nevertheless, after years of practical test and adjustments a complete success in the achievement of a full protection from infections in the vineyards with high epidemiological pressure has not been reached yet.
For a better understanding of the disease epidemiological pressure, we report the disease dynamics of *P. viticola* recorded over the last 11 years in two vineyards in the Treviso province (Venetian Region) together with the climatic data of the area.

The DOC Piave District. With about 25.000 hectars of cultivated vineyards, the Treviso province is one of the most important winegrowing areas in the Veneto Region. An important part of the winery production is represented by the the red wines produced in the plain areas along the Piave river (Consorzio DOC Piave, www.vinidelpiave.com). In this area, an extension service to support the vinegrowers in controlling the downy mildew, is operated since the early 80's. The service is based on the knowledge of *P. viticola* potential risk of epidemics, of climatic data and of the active mechanisms of fungicides. To afford a complete protection from *P. viticola* infections, many fungicide treatments are necessary. The protective schedule is mostly based on 2 "opening" treatments with ditiocarbammates, followed by 3-4 treatments using systemic fungicides (Figure 1).

Figure 1: Number of treatments against downy mildew distributed per year.

	May			June			July			August	Total
	decades			*decades*			*decades*			*decades*	
Products	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	
DTC*		1	(1)								1 - 2
Systemic				1	1	1	(1)				3 - 4
Copper						(1)	(1)	2	2	1	5 - 7
											10 - 13

* Ditiocarbammates

After the fruit-set stade (late June) 5-6 treatments with copper fungicides are distributed until early August. Thus, the total number of treatments adds up to 10-13 per year. A light protection against powdery mildew, based on sulphur and a few systemic products, mixed to the antiperonosporics, is usually sufficient to protect the bunches, as this disease is not a real risk in the area. A facultative treatment can be done on the bunches to protect them against *Botrytis cinerea*, but only on the cultivar more exposed to this disease.

The experimental plots. Epidemiological and climatic data have been recorded in two vineyards of Merlot cv, cultivated in Susegana (years 1999-2002) and Spresiano (years 2003-2009). The soil is of medium texture with alluvial skeleton and calcareous pebbles. The two sites are very close to each other and the climatic conditions are very similar. Other characteristics of the two vineyards are reported in Figure 2.

The climatic conditions. The climatic factors which have been considered in this study were: minimum and maximum daily air temperature, mean of four values (min, max, 8, 19), percentage of air Relative Humidity, daily rainfall and foliar wetness. The climatic data have been recorded using a mechanic weather station equipped with two termohygrometers, a thermometer with maximum and minimum temperature, a pluviometer and a pluviograph. The weather station was located in an open space, close to the vineyard.

Epidemiology. Regarding the epidemiological data, observations on the dynamic of the disease were carried out in untreated plots, used as reference thesis in fungicide evaluation trials. Usual dimension of the plots was 15-20 plants per 4 replications (total 60-80 vine per thesis). Observations were carried out weekly, starting from early May and ending in early August: 200 leaves and 150 bunches per replication were observed and classified into 7 disease classes: 0 (0% surface diseased), 1 (0,1-5%), 2 (5,1-10%), 3 (11-25%), 4 (26-50%), 5(51-75%), 6 (>75%). Disease values were calculated following the Townsend-Heuberger formula (I%I) and percentage of diffusion (I%D).

Figure 2: Main characteristics of the observed vineyards in the Treviso province.

Site	Year of establishment	Training system	Distance: m
Spresiano	1997	Sylvoz	1,4 x 3
Susegana	1975		3 x 3

Results

The area is one of the most rainy in Northern Italy (average rainfall, from 1977 to 2008: 1.147 mm/year, CRA-VIT data). The favorable climatic conditions to *P. viticola* in this area are represented by the abundance of rainfall and by the high relative humidity of the air during spring and early summer (Figure 3). Also important to the spread of secondary infections is the foliar wetness which is caused not only by rainfalls but by dew condensation in the nights and early mornings during the summer (Figure 4)(Blaeser, 1978).

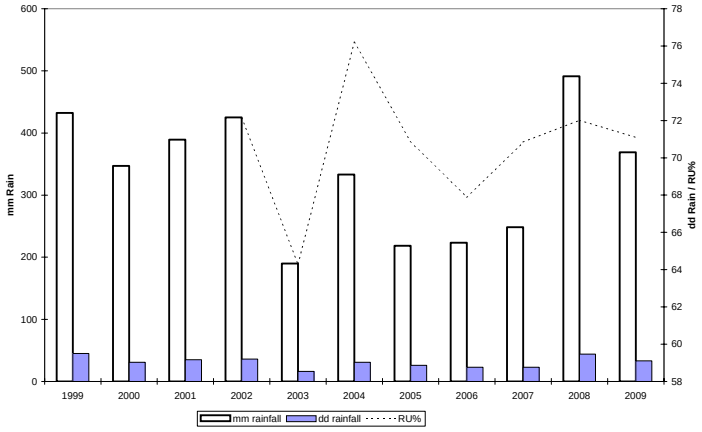

Figure 3: Rainfall (mm and days) and mean Relative Humidity (RU%) from the end of April to the end of July during the years of observation.

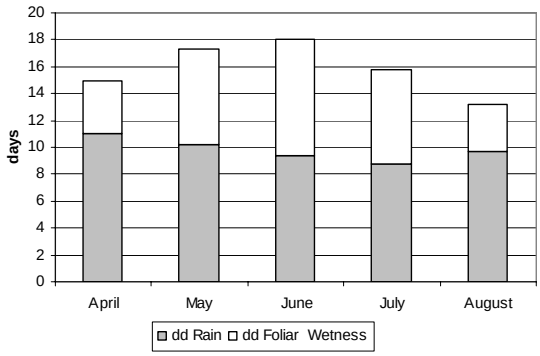

Figure 4: Days of rain and days of foliar wetness during spring and summer months (average 1999-2009)

This gives the fungus a wide possibility in establishing the first infections and eventually to spread over the vineyards. During the eleven years of observation most of the conditions necessary to establish foliar infections (10 mm of rain in 24-48 hours; 10°C of average temperature; 10 mm of length of shoots) were present in the first decade of May. The date of appearance of the first symptoms on the leaves ("oil spots") were observed 6-11 days after the day of infection. In most of the years (8 out of 11) the first "oil spots" were noticed in the second decade of May (Figure 5).

Symptoms on the grapes appeared later, 10-30 days after the observation of oil spots on the leaves. This is consistent with the observation of other Authors about the delay in symptoms appearance on bunches (Vercesi *et al.*, 2000). During the eleven years of observation, five years resulted in high or very high disease levels (1999-2002-2004-2007-2008), 4 years of middle intensity (2000-2001-2005-2009) and only two years in very low disease levels (2003-2006) (Figure 6 and 7).

The development of the disease - on leaves and bunches - was affected by the climatic conditions: the start of epidemics resulted to be associated with frequent rainfalls while high disease incidences were favored by early appearances of the first symptoms, high sensibility of vine herbaceous organs, foliar wetness during the nights and high levels of relative humidity of the air. A good correlation factor and a statistical significance was calculated between the cumulated rainfalls, from the end of April to the end of July, and the disease incidence (I%I) both on leaves and bunches (Figure 8).

Figure 5: Date of appearance of the first symptoms on leaves ("oil spots") and grapes (in italic) in the vineyard.

	May			June			July
	decades			decades			decades
Years	1	2	3	1	2	3	1
1999		14			*13*		
2000		18	*~30*				
2001		16	*30*				
2002		14		*5*			
2003					*12*		*5*
2004		14	*24*				
2005		19			*15*		
2006		15			*15*		
2007			31		*11*		
2008		13	*29*				
2009					19-*19*		

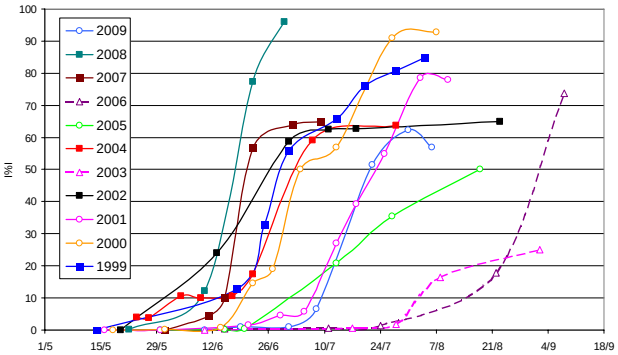

Figure 6: Development of disease infection on leaves (I%I).

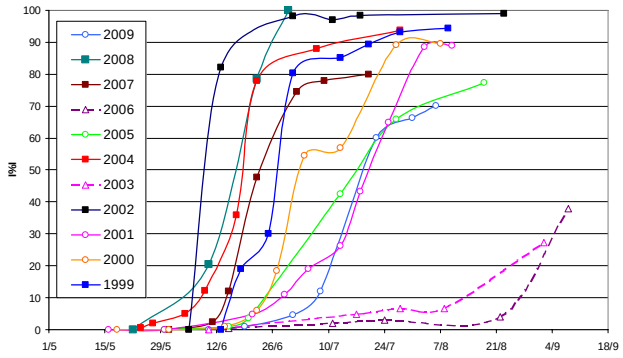

Figure 7: Development of disease infection on bunches (I%I).

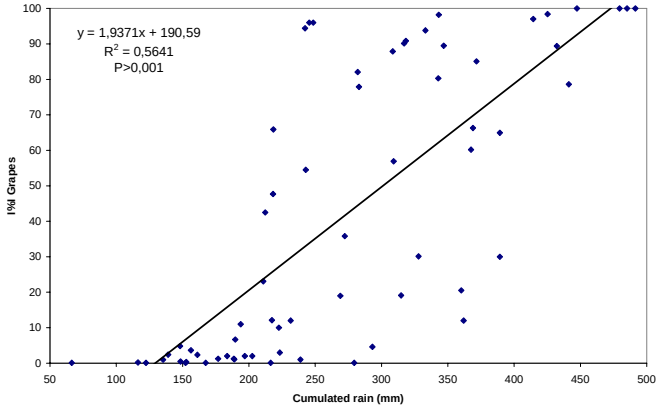

Figure 8: Regression of the cumulated rainfall from late April to late July vs. the disease incidence on grapes (I%I).

Delays in the disease diffusion in spring and summer could be caused by sudden changes in the air temperature: low temperatures cause a reduction in the fungus capability to grow while strong increases of temperature are ususally associated with lack of rain and low relative humidity levels. A part from 2003 and 2006, the final disease values were always very high, both in leaves and grapes. These observations confirm the high risk of *P. viticola* infections on the cv. Merlot in this area and the necessity of a careful and rational disease control during spring and summer.

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The Relationship between Environmental Factors and Grape Downy Mildew Epidemics in Shandong Peninsula Districts of China

Y. Jiye [a], L. Jianhua [a], W. Yuan[a], W. Zhongyue[b], L. Xinghong[a*]

[a]Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China, [b]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China
*corresponding author: lxh1962@yahoo.com.cn

Grape downy mildew cause by *Plasmopara viticola (*Berk. and Curt.*)* Berl. and de Toni.*,* is one of the most important grapevine diseases throughout most of the world. The pathogen can infect all parts of the plant, and cause severe damage. The traditional management method for the pathogen is to use chemical fungicides. To better understand the relationship between the environmental factors and epidemic of grape downy mildew, we monitored environmental factors and disease incidence of grapevine growth in Shandong Peninsula Districts.

Materials and methods

This research was carried out in Zhangyu Company Base Shandong Peninsula in 2009 on the grape cultivar is *Cabernet Sauvignon*. Ten to twenty leaves far away from test areas every week were selected to observe the disease pattern. After incubating in moisture for 12 hours, we recorded the sporangia incubation period once they were noted.

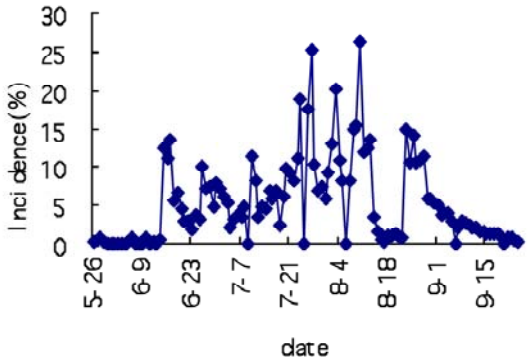

Figure 1: Daily disease variation of grape downy mildew in 2009.

One hundred of plants were selected for recording increasing infected leaves with a fixed five-point sampling method. The infected leaves with visible lesions were recorded. Finally, the incidence was calculated with the following formula: incidence= (investigated infected leaves/ investigated leaves)×100%.

The hourly changes of sporangium number in air were observed with portable spore traps (Plant Disease Research Laboratory of Popular and IPM, Hebei Agricultural University. Meteorological data were collected by automatic weather station (HOBO U30 NRC), including air temperature, relative humidity, rainfall, solar radiation, soil moisture, leaf moisture, wind direction, and wind speed.

When determining the outbreak date of disease, we could infer several important date of penetration period with incubation period. The period's environmental conditions were summed up, and the optimum threshold of environmental conditions can be determined. Analysis the data with stepwise regression analysis and determine the quantitative relationship among air spores, environmental factors and daily disease variation. Data was analyzed with Excel2003 and DPS.

Results and discussion

We recorded the incidence every day showed in Figure1, and inferred the penetration period with incubation period showed in Table 1. There are 7 peaks in grape growth season in Shandong Peninsula Districts.

Table 1: Disease outbreak date and infection period duration.

Disease Outbreak date	Incubation period	Infection period duration
Jun. 15~Jun. 17	7	Jun. 8~Jun. 10
Jun. 26~Jul. 1	6	Jun. 20~Jun. 26
Jul. 10~Jul. 11	6	Jul. 4~Jul. 5
Jul. 24~Jul. 27	12	Jul. 12~Jul. 15
Aug. 3	5	Jul. 28
Aug. 10	5	Aug. 5
Aug. 23~Aug. 28	10	Aug. 13~Aug. 18

The period of penetration and peak of spore numbers are not corresponding, which suggested that the climatic demands and infection demands are differences. The infection did not require abundant spores, while suitable conditions persisted.

We recorded the number by every two-hour and then average the numbers of several days. The dynamics is shown in Figure 3. The number of sporangia dispersion in day is more than that in night.

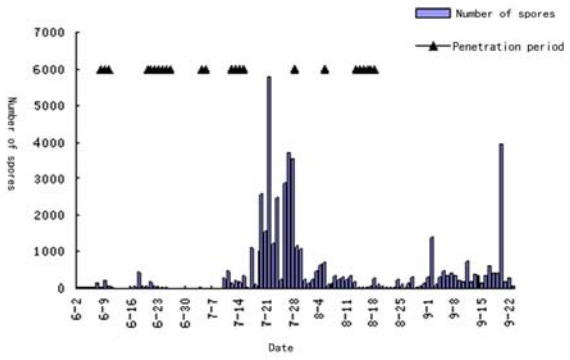

Figure 2: Downy mildew sporangia growth-season dynamics and penetration period.

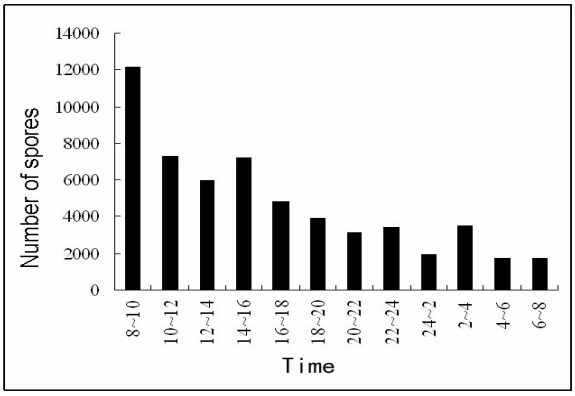

Figure 3:. Daily dispersion dynamics of Downy mildew sporangia in Shandong Peninsula of 2009.

The number of grape downy mildew flying sporangia was considered as the dependent variable, the relevant meteorological factors was considered as independent variables for stepwise regression to obtain the following regression equation.

Y= 36684.24-1198.36X1-170.81X2+8.55X3,

where Y is the average number of collected spores every two hours in the growing season, X1 is the average temperature of that time, X2 is the average humidity, and X3 is the average total amount of solar radiation. The equation is evaluate as the significant with test (The correlation coefficient R = 0.83, coefficient of determination R2 = 0.76, significance level P = 0.02 <0.05, F = 6.13). The result showed that grape downy mildew sporangia on dispersion dynamics and weather conditions are closely related, which affected by air temperature, relative humidity and solar radiation.

The temperature range is from the minimum temperature to the highest temperature in the peak of incidence period, so the difference may be greater than the mean temperature difference.

The incidence peak is considered as the dependent variable, relative factors (night relative humidity, average leaf water film daily, day and night temperature difference, spore volume) is considered as the independent variable, the following linear regression equation.

Y=-38.60-0.27X1+1.06X2-1.86X3,

where Y is the peak incidence, X1 is the leaf surface moisture, X2 is the night-time humidity, and X3 is the temperature difference between day and night.

The equation is evaluated as significant with the correlation coefficient R = 0.9990, coefficient of determination R2 = 0.9980, significance level P = 0.0001 <0.01, F = 505.68). The partial regression coefficient t-test is also significant. [R(Y , X1)=0.0011<0.01 , R(Y , X2)=0.0001<0.01 , R(Y , X3)=0.0023<0.01].

The results showed that three kinds of environmental factors on the peak incidence of downy mildew play a major role, its associated degree of relationship is X2> X3> X1, and then night-time humidity on the downy mildew occurred is the greatest impact factor, followed by day and night temperature difference, leaf surface moisture is the minors factor. The amount of spores and the incidence rate has not shown significant correlation, which may be due to the incidence of all samples exceeded the critical amount of spores.

From Table 2, we can also infer that temperature range of downy mildew outbreak is 15.94~38.30℃, the minimum relative humidity is 72.03% at night, which is the critical condition for disease outbreak.

Table 2. The relationship between the peak incidence of grape downy mildew and environmental conditions in its incubation period.

Ratio of disease (%)	Temperature range (℃)	Leaf surface humidity (%)	Evening Humidity (%)	Temperature difference between day and night (℃)	Sporangium scattering volume (个)
9.82	15.94~34.41	30.45	73.64	11.44	49.50
7.02	18.53~38.30	35.46	72.03	11.21	63.56
9.85	18.27~32.15	49.29	75.86	9.92	75.64
20.60	18.91~29.72	63.49	87.40	8.42	1160.53
20.05	20.20~29.62	75.16	90.27	8.96	1467.67
20.86	22.80~31.08	77.97	91.56	8.81	1042.60
14.80	16.15~31.00	68.81	86.25	10.05	78.00

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Acknowledgement

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Session 4: Disease management, disease economic impact, desision model, forecasting models, fungicide efficacy, biocontrol

Integrated grapevine powdery and downy mildew management in south eastern Australia: Evaluation of the impact of long term research and development

R.W. Emmett[a], J. Edwards[b], M. Barlass[c]

[a] Biosciences Research Division (BRD), Department of Primary Industries (DPI), PO Box 905, Mildura, VIC 3502, Australia; [b] BRD, DPI, Private Bag 15, Ferntree Gully Delivery Centre, VIC 3156, Australia; [c] BRD, DPI, GPO Box 4440, Melbourne, VIC 3001, Australia

In Australian viticulture, powdery mildew is a widespread persistent disease that can cause major crop losses and decrease wine quality. In individual vineyards of susceptible varieties, entire crops can be lost if control measures are inadequate. Wineries reject grapes from vineyard patches with more than 3% bunch disease severity. Grapevine downy mildew can also reduce production significantly when control measures are inadequate in wet seasons. Generally one in 10 seasons has weather favourable for severe downy mildew epidemics. Substantial expenditure on control programs also contributes to the high annual cost of powdery and downy mildew.

The evaluation of benefits arising from investments in research and development (R&D) programs is important for horticultural industries and governments to support and justify future investment. Major co-investors in horticultural R&D in Australia include industry and Federal and State governments. Rural industry research corporations (e.g. the Grape and Wine Research and Development Corporation, GWRDC) are responsible for the investment of industry funds collected from levies on production and matched funds from the Australian government. State government agencies (e.g. the Department of Primary Industries in Victoria) also invest State government funds in R&D projects that will provide benefits to the State.

This paper examines the impact of long term investment in a R&D program on integrated powdery and downy mildew management in vineyards in south eastern Australia between 1980 and 2008, particularly R&D co-funded by the Department of Primary Industries in Victoria (DPI Vic). Other major co-investors included the South Australian Research and Development Institute (SARDI), the Cooperative Research Centre for Viticulture (CRCV) and GWRDC. The series of R&D projects in the program covering research, development and extension involved researchers from Australia and other countries, especially the USA.

Methods

Industry situation before the R&D. Information on the control of powdery and downy mildew in vineyards and its efficiency and effectiveness before the R&D commenced was compiled.

R&D program outputs. Key outputs from projects in the R&D program, especially those related program outcomes were identified.

Industry situation after the R&D. Information on the efficiency and effectiveness of powdery and downy mildew management in vineyards after completion of the R&D program was compiled.

R&D program outcomes. Perceived outcomes or impacts from the R&D program were determined by comparing the situations before and after the R&D.

Economic evaluation of the R&D. This part of the study has commenced and will be completed by June 2010. The basis of social outcomes for the industry, community and environment will be examined to establish *bona fide* links to R&D project outputs and determine which projects and investments were directly related to the outcomes. Positive and negative impacts arising from the R&D at the industry, community and environmental level will be identified.

Rates and levels of adoption of R&D outputs over time in relation to linked outcomes will be determined and confirmed through consultations with researchers and extension specialists, and then with producers and industry representatives with long term experience. Information on adoption of outputs will also be compiled from surveys of industry practice or other records (e.g. vineyard spray diary summaries).

Economic analyses will be conducted to quantify R&D economic performance indicators, particularly the benefit-cost ratio (BCR), net present value (NPV) and internal rate of return (IRR) for financial investments in the R&D. The economic analyses will involve establishment and comparison of 'with' and 'without R&D' scenarios, each with identified benefits and costs. Important qualitative benefits from the R&D (i.e. non-direct, non-quantifiable benefits in this evaluation) will also be identified and described.

Results

Industry situation before the R&D. Generally grape growers had some knowledge of factors affecting powdery and downy mildew activity and of conditions for disease development in vineyards and most growers applied extensive spray programs as 'insurance' to prevent crop loss.

Powdery mildew occurred annually in many vineyards and grape yield and quality losses were common, regardless of the application of spray programs, especially on highly susceptible vine cultivars when warm humid weather favoured disease development. Vine disease severity thresholds used by wineries for crop rejection or down-grading were often inconsistent and/or loosely applied.

Seasonal incidence and severity of downy mildew in vineyards varied with the weather. Major yield losses occurred in some vineyards in some seasons, regardless of the application of spray programs, when periods of wet weather in spring and summer favoured disease.

Most fungicide sprays for the prevention of powdery and downy mildew in the 'average season' were applied according to calendar date or vine growth stage, regardless of seasonal weather conditions and the presence or absence

of disease. In the early to mid 1980s, most grape growers routinely applied at least 5-6 fungicide sprays each season. By the mid to late 1990s, at least 8-9 sprays (range 4-14 sprays) were applied at average intervals of 14 days (range 7-21 days). While these spray programs usually provided some disease control, they were inflexible, inefficient, costly and often ineffective because of inappropriate spray timing and use of fungicides.

On average, 8-9 sprays were applied for powdery mildew control on susceptible varieties each season in the 1990s, mostly from 4 weeks after bud break (modified Eichhorn and Lorenz growth stage 15, E-L 15; Coombe, 1995) to veraison (E-L 35). From 7-8 sprays were applied for downy mildew control, mostly from 4 weeks after bud break (E-L 15) to when berries were pea-size (E-L 31). Some growers applied sprays up to 4 weeks before harvest. Others applied a lower number of sprays, but often at intervals of more than 14 days. Different fungicides applied for powdery or downy mildew control were tank-mixed. Sulphur and copper fungicides were widely used for the control of powdery and downy mildew, respectively.

Fungicide residues in grapes and wine, inadequate fungicide performance because of inconsistent disease control and fungicide resistance were significant risks arising from poorly designed and applied spray programs and excessive use of fungicides by some grape growers.

Factors that increased the need for grape growers to adopt improved management practices in the period before and during the R&D included:

(1) Increased vine vigour and canopy size because of the increased use of rootstocks and more accessible pressurised irrigation;

(2) Increased vine disease susceptibility because of the wider use of more susceptible varieties (e.g. Chardonnay) and the increased use of over or under vine spray irrigation;

(3) The periodic occurrence of seasons with weather that promoted disease epidemics, especially downy mildew.

R&D program outputs. Key outputs from projects in the R&D program included those noted below.

Powdery mildew

(1) Increased knowledge of powdery mildew development in vineyards (inoculum sources, primary infection, disease spread, vine variety and vine tissue susceptibility, effects of weather, vine canopy and irrigation management).

(2) Procedures developed for monitoring vineyard disease incidence and severity, assessing vineyard disease potential and assessing disease incidence thresholds for initial spray application.

(3) Disease simulation and risk assessment models developed overseas assessed for use, but found to have only limited applicability in Australian vineyards.

(4) Spray programs closely aligned with seasonal pathogen activity (e.g. early season '2-4-6' and 'look first' spray program options) developed for more efficient and effective disease control.

(5) Long term spraying strategies developed to reduce overwintering inoculum (bud infection, cleistothecium development) and powdery mildew incidence and severity in vineyards.

(6) A computer based 'vineyard spray program evaluator' developed for potential on-line use and for analysis of spray records in spray diaries or planned spray schedules to indicate spray program disease control efficiency.

Downy mildew

(7) Increased knowledge of downy mildew development in vineyards (inoculum sources, primary infection and secondary infection, disease incubation and spread, vine and crop susceptibility, influence of weather conditions).

(8) Procedures developed for assessing disease presence in vineyards and monitoring disease incidence.

(9) A computer-based downy mildew disease simulation model (DModel) developed for assessment of weather data to indicate primary and secondary infection events, incubation period and disease appearance.

(10) A risk-based spray program based on predictions of disease appearance (DModel) and appropriate use of post and pre-infection fungicides developed for more efficient disease control.

(11) A low cost automated weather station (Model T Met Station) designed to collect and process weather data and provide on-farm predictions of downy mildew infection events.

Integrated disease management

(12) Integrated risk-based spray programs for powdery and downy mildew control widely evaluated in commercial vineyards, especially in Victoria and South Australia.

(13) Increased knowledge of fungicides and recommendations for their optimal use in integrated pest and disease management programs (e.g. sulphur for powdery mildew control, phosphorous acid for post infection control of downy mildew, strobilurin fungicides around flowering and up to 4-5 weeks after berry set for powdery and downy mildew control).

(14) Computer-based, grapevine powdery and downy mildew management models developed and incorporated into the CRCV AusVit^{TM} computer-based decision support system for processing weather and disease activity data and developing disease warnings and spray recommendations extended through regional disease and pest alert services (e.g. CropWatch SA^{TM}).

Industry situation after the R&D. Most grape growers are aware of the key factors that influence disease behaviour and driving good disease management. They use published and online information to support the development and implementation of successful disease management programs when required. This includes disease identification and management guides, 'rules of thumb' to emphasise key management strategies (e.g. '2-4-6' for powdery mildew control in warmer districts, '10:10:24' for downy mildew primary infection), disease alert services and/or commercial vineyard monitoring services (some regions), and improved directions for spray application and fungicide use for disease control on fungicide labels.

Disease management tools are also used by vineyard managers and viticulture consultants where required. These tools include vineyard disease monitoring and assessment procedures, disease incidence/severity thresholds for spray application, and procedures for assessing vineyard disease potential and selecting appropriate management strategies for powdery and downy mildew, and the disease prediction model and risk-based management system for downy mildew.

Powdery mildew control programs with early season sprays and several types of fungicides are widely used. These provide good disease control and avoid the need for late season sprays that can promote fungicide resistance and increase the risk of chemical residues on the crop. While many grape growers achieve good disease control with 4-6 sprays each season, most apply 6-7 sprays from 2

weeks after bud break (E-L 12) to 4 weeks after berry set (pre-bunch closure, when berries are pea size, E-L 31).
Risk-based spray programs are widely used for downy mildew control. Modified routine spray programs are used in some vineyards where logistics limit the use of risk-based programs. In the latter programs, sprays for downy mildew are tank-mixed with powdery mildew sprays. In risk-based programs, spraying is mostly aligned with seasonal frequency of downy mildew primary infection periods. In some districts (e.g. Sunraysia), no sprays are required in 15% of seasons when there are no primary infection periods while a full spray program with 6-8 sprays is required in another 15% of seasons when there are four or more primary infection periods and conditions favour severe disease epidemics (Magarey *et al.*, 1993). In modified routine spray programs, usually 4-5 sprays are applied, mostly in the period from 2 weeks after bud break (E-L 12) to when berries are pea-size (E-L 31).

R&D program outcomes. Grape grower knowledge of powdery and downy mildew in vineyards has substantially increased because of the uptake of information produced and/or delivered by the R&D program coupled with other approaches to educate growers. In an array of mechanisms, more extensive information is available to support the development and implementation of successful disease management programs.
Most spray programs applied for the control of powdery and downy mildew are more effective and efficient, risk-based and better designed for the situation and purpose. The latter includes wine, dried and table grape production, and organic and low input viticulture. Design of the programs takes into account key factors, particularly current seasonal pathogen activity and disease risk, seasonal weather conditions, and vine susceptibility and cultural practice. Fungicide selection in these programs is also more appropriate, taking into account fungicide type, function, purpose, withholding period and requirements for fungicide resistance management. Generally vineyard managers are better equipped to design and apply more adaptable, knowledge-based disease management programs where the use of different types of fungicides (including new and soft fungicides) is matched to different levels of disease potential.
Powdery mildew occurrence and crop loss in vineyards each season has decreased markedly. In the Sunraysia and Riverland districts, for example, incidence of vineyard patches with grape yield and quality losses has declined from around 10% before the R&D to around 1% after the R&D because of the application of more effective disease management programs. Yield losses caused by downy mildew rarely occur because control programs are aligned with disease risk and are more reliable and sustainable.
On average, the number of sprays applied for powdery mildew control each season has been reduced by two (8-9 to 6-7 sprays, 23-25% reduction). Where growers are applying modified routine spray programs, the number of sprays applied for downy mildew control each season has been reduced by three (7-8 to 4-5 sprays, 37-43% reduction). The reduction in sprays for downy mildew control is even greater where growers are applying risk-based sprays aligned with the occurrence of infection periods, especially those for primary infection. In the Sunraysia district around Mildura, around 50% of seasons have either one or no primary infection periods (Magarey *et al.,* 1993) requiring either one or no sprays with a post infection fungicide for disease control. Grower spraying practice is now more aligned with these events.
The risk of fungicide residues in grapes and wine, and inadequate fungicide performance has been reduced substantially because of improved spray program design, the shift towards spraying for disease control earlier in the season, reduced dependence on late season sprays and increased grower knowledge and application of fungicide withholding periods, fungicide resistance management practices and improved spray technology.

Economic evaluation of the R&D. Results from this part of the study are planned for early July 2010 after the evaluation has been completed.

Acknowledgements
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Global economic importance of Grape Powdery and Downy Mildew protection

D. Steiger

Portfolio Management Fungicides, Global Product Manager Bayer CropScience, Alfred-Nobel-Strasse 50, 40789 Monheim am Rhein, Germany, e-mail: dominique.steiger@bayercropscience.com

This analysis of the economic aspects of grape powdery and downy mildew protection is based on Bayer data taken from the professional crop protection market at farmer level. Prices and values are just an indication and "treated hectares" refers to the total number of treatments during the season. Beside the "pure" powdery mildew and downy mildew treatments, which in 2008 represented 20.5 and 19.4 million treated hectares respectively, there is also the so called "mixte" segment of ready-mixed products which are simultaneously active against both powdery and downy mildew. This segment represents an additional 1.4 million of treated hectares.

Between 2005 and 2008, downy mildew was the winegrower's major crop protection investment, representing 33% of the total grape protection expenditure. Powdery mildew comes second with about 22% of the total when the "mixte" products only account for 4. Other plant protection investments represent 9% for botrytis, 16% for insecticides and 14% for herbicides.

The efforts taken by winegrowers to protect their vines against downy mildew vary considerably from one year to the next, especially in Europe, were downy mildew investments can fluctuate by up to 20% depending on the disease pressure. The expense on powdery mildew and mixte products has, in the rest of the world been, much more stable over recent years.

European countries are by far the major users of downy and powdery mildew products accounting for more than 90% of the total downy mildew product consumption and more than 75% of powdery mildew in 2008.

The twelve major countries for downy mildew protection are France, Italy, Spain, Portugal, Brazil, Germany, Australia, Romania, Austria, Slovenia, Bulgaria and China. After more than 130 years copper products are still the most used products for the control of downy mildew, followed by dithiocarbamates, Fosetyl mixtures, anilides mixtures, CAA mixtures and cymoxanil mixtures. All in all, the winegrower has access to about seven different modes of action to prevent downy mildew attack in vines, not including the multisite compounds (mainly copper, dithiocarbametes and phtalimides) and the specific fungicide Fosetyl-Al, a Natural Defense Stimulating compound. On average the winegrower spend 22 € to protect one hectare of vines against downy mildew. However, prices vary considerably from 10 €/ha for a low-cost contact products to more than 45 €/ha for a more sophisticated product with longer treatment interval.

For powdery mildew the twelve main countries are France Italy, USA, Spain, Chile, Portugal, Germany, Turkey, Greece, Austria, Australia and South Africa. The powdery mildew segment consists on one hand of multisite compounds, mainly sulphur and dinocap (36 % of the total) and on the other of six different categories of single site mode of action: SBI1, QoI, Quinoleines, SBI2, Benzophenone and SDHI. The cost per hectare is on average 11 € for a powdery mildew treatment, starting at 7 € for a basic sulphur product and rising to more than 20 €/ha for a combination of different modes of action which minimise the risk of resistance development.

The "mixte" segment appeared some years ago because of the dual activity of some QoI against both downy and powdery mildew. The use of real built-in ready-mixed solutions, associating a powdery active and a downy active is very limited and represents about 20% of the "mixte" products and only 1% of the total downy treatments.

This historical lack of interest from the winegrower for a complete downy and powdery mildew solution is certainly linked to the biological differences between downy and powdery development conditions. In most cases the winegrower prefers to tank mix a downy and a powdery mildew product together. Depending on the weather conditions and the respective downy and powdery disease pressure, he will choice the most adapted downy or powdery mildew product and then decide whether or not to associate it with the right powdery or downy mildew product. From the economical perspective there is no real difference, he will pay for a "mixte" product from 25 to 50 €/ha which is on par with the total cost of a downy mildew product plus a powdery mildew product.

Testing a decision system for Integrated Protection against Mildews the vine-grower, the adviser, and the computer model

O. Naud[a], L. Delière[b], P. Cartolaro[b], B. Léger[c]

[a]Cemagref – UMR ITAP, BP 5095, 34196 Montpellier Cedex 5 - France; [b]INRA-Bordeaux UMR INRA-ENITA 1065 Santé Végétale – ISVV, BP81, 33883 Villenave d'Ornon – France; [c]Arvalis - Institut du végétal, station de la minière, 78280 Guyancourt - France.

In 2005, continuing work they had done on a disease per disease basis, a team of phytopathologists from INRA sketched a combined decision system to manage both powdery and downy mildews at the same time. In 2007, as a result of a collaboration with computer and automation scientists from Cemagref, this decision system named GrapeMilDeWS, or "Mildium" in French, was modelled in the Statecharts formalism, after elicitation of the experts' knowledge. It was then called a decision workflow, because there was great similarity in the building of the model with what is done in workflow modelling e.g. for business process management within service-oriented companies.

There have been recently a few attempts to use workflow modelling for farm management (e.g. Guan et al, 2008; Wolfert et al, 2009). Yet, we think that many agronomists and phytopathologists are not familiar with these concepts and tools. Workflow modelling is about the modelling of how and when work should be carried out, especially when there are electronic traces of the flow of work (tasks) performed within an organization. The purpose of this paper is to demonstrate how workflow modelling, and formal paradigms such as automata and discrete event systems, may contribute to the design of decision systems for protecting grapevine against powdery and downy mildews in a sustainable agriculture framework. The outline of this paper is as follows. We will review the needs of the vine growers and their advisers, and point out the importance of sound tactics for the management of grapevine mildews. We will then briefly recall the main principles of GrapeMilDeWS. GrapeMilDeWS has been detailed and explained in (Léger & al, 2008; Léger & al 2010). The initial modelling process and knowledge elicitation has been described in (Léger & Naud, 2009). We will afterwards argue that the value of experimenting GrapeMilDeWS is not limited to simple performance testing. Using a protocol designed on the basis of a decision workflow model, it should be possible to consolidate facts into operational epidemiological knowledge about plots "with few or limited number of treatments". We will also suggest that the model of the decision system can evolve according to design stage, and model behaviour analysis.

Context and background

According to governmental initiatives such as "Grenelle de l'environnement" and "Ecophyto 2018", the political roadmap in France is set towards reducing pesticides use by a factor of 2 "whenever possible". This puts forward research needs in two directions: designing potential new solutions, and evaluating existing as well as novel solutions in regards to the three criteria of sustainability. The GrapeMilDeWS decision system is a research contribution to the question that we can formulate as follows: "can we do more about specific control ('*protection raisonnée*', the decision part in an Integrated Crop Protection Strategy) of grapevine against downy and powdery mildews ?"

The Grower, the Adviser, and the decision system

On the one hand, the growers know that both grapevine mildews are polycyclic and that the secure way for reaching their production objectives is to act preventively against contaminations and disease propagation. On the other hand, they are also aware of the social expectations for moderate use of pesticides and have to consider spraying only "when necessary". In this regard, a grower is expected to (i) estimate his risk, which is local in space and time, and (ii) decide the appropriate sprayings and justify each of them. Over the production year, the issue is mainly tactical, which means that organisational matters are included in the economical problem.

In order to reach the above-mentioned objectives, extension services provide the grower with two kinds of advices. The first type of assistance is to estimate the risks of epidemics and justify this estimation to the grower. It is yet provided in France on a region basis by "crop health bulletins" which report about contaminations detected during plot surveys. No practical advice about necessity to spray is given in these bulletins because this second way of assisting the grower has been strictly separated from crop health monitoring. Advice about necessity to spray is provided by a set of actors, professional bodies and companies. It should be noticed that the scale and the number of criteria included in the advice do matter. Our hypothesis is that there are possibilities of progress in the advice about whether to spray or not spray by studying relations between local risk, estimation of local risk evolution, and crop protection tactics. From this hypothesis, we draw that experimenting GrapeMilDeWS can provide knowledge about the behaviour of the grapevine pluri-pathosystem in situations with "reduced number of treatments".

GrapeMilDeWS is a decision workflow. It includes description of tactics to support the general strategy that epidemic levels should be assessed early, in order to keep control with a limited number of treatments. As expected in an IPM framework, at harvest time, the net profit criteria (i-e fruit quality and quantity) is of higher concern than the quantity of disease spots on the leaves, provided the latter is small enough to avoid inter-annual inoculums accumulation effects.

GrapeMilDeWS at a glance

The decision system is organised as a sequence of 7 stages that cover the period from bud-break to ripening. Except stages 0 and 6, each stage is typically and roughly 2 weeks long and its start is labelled with phenology indications. The sequence of stages is summed up in table 1. Phenology is indicated in the BBCH scale.

Table 1: Stages of GrapeMilDeWS

Stage	BBCH start	Target diseases
0	08	Downy M (optional)
1	15-17	Downy M (optional), Powdery M (mandatory)
2	18-20	Downy M (optional), Powdery M (optional)
3	61-65	1 combined spraying (T3) against both Downy and Powdery Mildews
4	Once T3 is no more active	Downy M (optional), Powdery M (optional
5	T3 + 4 weeks (76-77)	Downy M (optional), Powdery M (optional
6	81-83	Downy M (mandatory copper treatment)

Stages 1 and 2 are each preceded by a plot survey, where frequency of contaminated stocks (leaves) is estimated for a set of 100 stocks evenly distributed in an area with approx 1000 stocks. Stage 5 is preceded by a third survey, for which powdery mildew contaminations are monitored over 5 bunches per sampled stock. Estimation of downy mildew follows the same protocol as defined in prior plot surveys.

Table 2 enumerates the qualitative indicators derived from these plot surveys. It is important to notice that these surveys are not intended to provide a detailed evaluation of the dynamics of the epidemics, but are used to decide about the necessity to spray against a given target.

Table 2: Plot survey indicators of GrapeMilDeWS

Survey	Indicators	Qualitative values and thresholds
C1	M	M- M+ (≥1% stocks) M++ (≥10% stocks)
	O	O- O+ (≥2% stocks) O++ (≥20% stocks)
C2	M	M- (≤10% stocks) M+ (2%≤M≤50% stocks) M++ (≥50% stocks)
	O	O- (≤20% stocks) O+=O++ (≥20% stocks)
C3	M	M- (≤10% stocks OR C3≤C2 *unless C3=100%*) M+ (2%≤M≤50% stocks) M++ (≥50% stocks)
	Og	Og- (≤20% bunches) Og+=O++ (≥20% bunches)

Besides plot surveys, GrapeMilDeWS relies very much on two other bioclimatic indicators. One (called PA) is the presence of rain forecast (for a given expected date) and the other (called ILM) is an expert interpretation of local downy mildew risk. ILM relies on surveys provided by extension services on local networks of plots and on bioclimatic risk models.

We will not provide here technical modelling details of GrapeMilDeWS but recall some major facts. The elicitation of the experts' knowledge was performed with the support of Statechart diagrams, to provide the experts with direct control over the modelled decision sequences and their logics (Léger & Naud, 2009). The experts recognized that the Statechart specification model was fair to their original design. The model behaviour was also assessed by simulation. The simulated behaviour was checked against actual decision behaviour of the experts (Léger, 2008). This verification showed that the model was very satisfactory and could be used as reference when establishing the GrapeMilDeWS experiment protocol to be distributed to a number of people. Yet, comparison of decision simulated from the model and decision made by experts showed evidence of the phenomena explained hereafter. For management of resources, which include human workforce to perform plot surveys, it is necessary to specify the level of flexibility in the delay between decisions and actions. It may also be worth to take anticipation behaviour into account, because anticipation makes management easier. Yet, weather forecasts are not very stable when it comes to predict rainfalls more than 3 days in advance. This means that anticipated decisions may have to be revised.

Crop protection performance

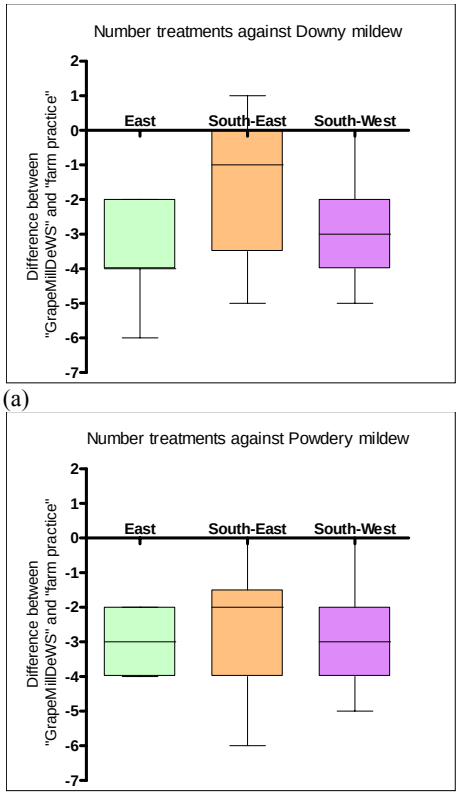

Figure 1: Reduction of treatments provided by GrapeMilDeWS in 2008 and 2009.

Figure 1 shows how many treatments were spared by GrapeMildeWS in comparison to the usual practices in the farms where GrapeMildeWS was experimented. The box-plots describe 39 cases in 2008 and 2009 (7 cases from the East, 8 cases from the South-East, 24 cases from the South-West of France). The reduction is effective. It should be noticed that 2008 and 2009 were years with high downy mildew pressure in the South-Western region. For information about GrapeMilDeWS' crop protection

performance in 2008 and 2009, figure 2 shows data on the relation between severity on bunches (sum of severities for downy and powdery mildews) and severity on leaves. The problematic points: above 30% severity on bunches, occurred in 2008 in Bordeaux region with a very early and fast start of the downy mildew epidemics. The 2008 protocol imposed an optional C0 plot survey before any treatment in stage 0, or waiting until C1. This procedure delayed the first treatment in several cases. The protocol for stage 0 was modified in 2009.

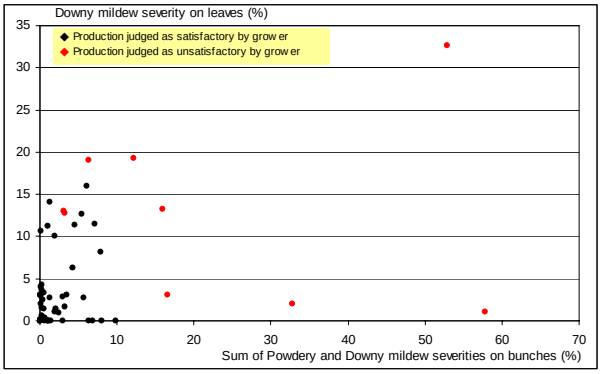

Figure 2: Severity on grapes and leaves for 56 cases of GrapeMilDeWS experiment in 2008 and 2009.

Analysis of crop protection tactics on downy mildew

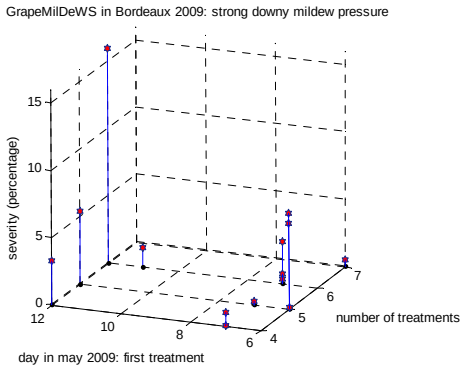

Figure 3: Analysis of performance of GrapeMilDeWS in Bordeaux region in 2009.

Figure 3 provides an example of analysis that can be conducted on experimental data. In this figure, it is checked, for a given region and year, whether the sum of severities regarding powdery and downy mildews depends on the number of treatments (y axis) and on the date of first downy mildew treatment (x axis). It is important to check this latter data because lack of protection against a significantly contaminating rainfall makes later protection more difficult. The figure contains only a few points and should not be interpreted as statistics but as the starting point of a diagnostic. One case with first treatment on May 12th appears to be unsatisfactory with more than 15% severity. In this case and this period of time, ILM could not be interpreted daily and was assessed solely according to past events, i-e with a lack of anticipation. Once the risk information was updated on May 11th, the spraying could not be organised before a significant rainfall on the afternoon of the same day. Because GrapeMilDeWS limits the number of treatments, it has best performance under high epidemic pressure with careful anticipation.

It can be also noted from figure 3 that on some cases from Bordeaux region in 2009, the number of downy mildew treatments could be limited to 4 with satisfactory results. This shows that GrapeMilDeWS responds properly to specific cases even in difficult years.

The figure suggests that for difficult situations, the number of required treatments oscillated between 5 and 6 treatments. The 6 treatments cases did not provide enhanced results when compared to the 5 treatments cases.

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A Bio-Economic Model to Evaluate and Compare Different Protection Strategies Against Grapevine Downy and Powdery Mildew

P. Leroy[a], P. Cartolaro[b], L. Delière[b], J.P. Goutouly[c], M. Raynal[d], A. Ugaglia[e]

[a]INRA ALISS, UR 1303, 65, bd de Brandebourg, 94205 Ivry-sur-Seine Cedex, France, [b]INRA Santé Végétale, UMR 1065, ISVV, BP 81, 33883 Villenave-d'Ornon Cedex, France, [c]INRA EGFV, ECAV, UMR 1287, ISVV, BP 81, 33883 Villenave-d'Ornon Cedex, France, [d]IFV Bordeaux Aquitaine, 39, rue Michel Montaigne, 33290 Blanquefort, France, [e]INRA GAIA, USC 2032, ENITA de Bordeaux, 1, cours du Général De Gaulle, CS 40201, 33175 Gradignan Cedex, France

Treatments against downy and powdery mildew represent a large part of the total treatments on French vineyards. Societal evolutions question producers about their practices. "Mildium", a new decision process, has been designed and experimented by INRA-Bordeaux to reduce the use of agrochemical products. The main issue is how to induce the producers to change their practices? The key factor is the economical risk linked with the damages on yield due to these pathogens. To complement this new approach, we have developed a bio-economic model[1]. The purpose of this model is to integrate the different aspects of the problem (agronomical, phytopathological and economical); to evaluate and compare the technical and economical risks associated with different treatment strategies in different contexts (agronomic, climatic, pressures of pathogens).

This model works at plot and daily levels.
We try to simulate the actions depending on the protection strategy (treatments, observations), to assess the damages on leaves and yield due to pathogens with different climatic scenarios (on the basis of registered climatic data), Furthermore, we assess the economical consequences taking into account the economical data (sale price of grapes action costs) and the mode of regulation in AOC[2] areas. On this basis, we can build statistics on the technical and economical results (Figure1) and analyse risks.

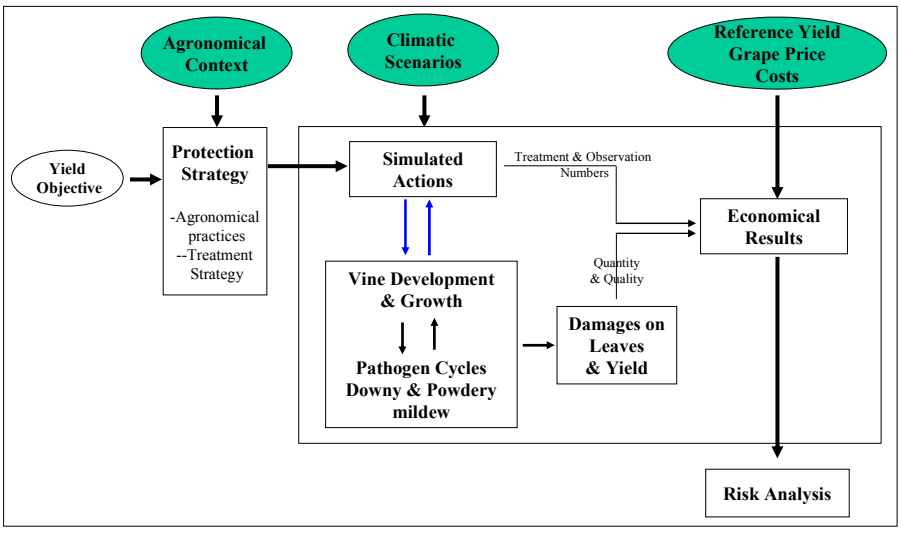

Figure 1: Principles of the bio-economic model

About the agronomical aspects, we use STICS-Vigne model (Garcia de Cortazar Atauri, 2006). It gives us, for each climatic scenario, the growth of the leaf area, the dates of the phenological stages of the vine, and the growth of the grape yield, depending on the vineyard structure, objectives, and management practices.

Damages on leaves

We take into account three processes: (i) Ontogenic resistance, considered as an age of the leaf area to get this resistance; (ii) Contamination by the pathogen; (iii) Protection by the treatments. Thus, daily, the total leaf area (LA_T) is splitting up into four exclusive compartments: resistant area (LA_R), contaminated area (LA_C), protected area (LA_P), and, the complement, that represents the area susceptible to be contaminated, as: $LA_S = LA_T - LA_R - LA_C - LA_P$.

The cycle of the pathogen is modelised by: a latent period as a temperature dependent function (Blaise & Gessler, 1992; Calonnec & al, 2008); a sporulating period characterised by a duration and a capacity function (1 to 0).

The primary contaminations are not directly modelised. They are assumed as scenarios defined by a rule: after an initial phenological stage, an event of primary contamination is possible if daily rainfall and temperature thresholds are reached; maximum number of events, and their level (in terms of contaminated area), are also rule parameters.

The formulation of secondary contaminations is inspired from Blaise & Gessler (1992).
$\Delta LA_C(t) = K_L(t) \cdot LA_{Spor}(t) \cdot LA_S(t) / LA_T(t)$,
where $LA_{Spor}(t)$ represents the sporulating area, and $K_L(t)$ a coefficient that may depend on climatic conditions: for downy mildew, the necessary humidity conditions for spore germination are modeled through a daily rainfall [RF(t)] threshold :
if $RF(t) >= RF_{Threshold}$, then $K_L(t) = K_L$ or else $K_L(t)=0$. For powdery mildew, $K_L(t) = K_L$.

Treatments effects are only considered as a protection of the leaf area, without any effects on the pathogen cycle. They are characterised by a duration, a maximum level, and a curve of efficiency.

[1]This model has been developed under the "Vin" project of the ANR unifying program "Agriculture & Développement durable".

[2] AOC: Appellation d'origine contrôlée.

Damages on grapes

The development mechanisms of the pathogens on grapes are not directly modeled.

Depending on the potential growth of the grape yield given by the STICS-Vigne model, we evaluate loss on yield through three processes : (i) effect of the pathogens, with, as indicator, the sporulating area [$LA_{Spor}(t)$], and a contamination coefficient on grapes [$K_Y(t)$]; (ii) a sensivity function for grapes [$Sens_Y(t)$] depending on the number of days since flowering ; (iii) a protection function for the last treatment (at T day) characterised, too by a duration, a maximum level, and a curve of efficiency [$Prot_Y(t,T)$].

Therefore, if $Y_{Pot}(t)$, $Y(t)$ and $Y_{Loss}(t)$ are respectively the potential yield, the current yield and the loss of yield at t day we assume that:

$$\Delta Y_{Loss}(t) = Y(t-1) \,.\, [K_Y(t) \,.\, LA_{Spor}(t) \,.\, Sens_Y(t)] \,.\, [1 - Prot_Y(t,T)]$$

$$Y(t) = Y(t-1) \,.\, [1 + \Delta Y_{Pot}(t) / Y_{Pot}(t-1)] - \Delta Y_{Loss}(t)$$

Treatment Strategies

At the moment we can represent two types of strategies:

- Systematic strategy, characterised by phenological stages for beginning and ending and a frequency for treatments.
- "Mildium" strategy[3] (Cartolaro et al, 2007): we represent the different steps of observations and decisions as described in the method. But, in this method indicators are mainly based on frequency thresholds on vine stock, while our model only estimates severity indicators. We have estimated the thresholds in terms of severity with experimental results in 2007.

Calibration of the phytopathogical sub model

Fist, we have calibrated the phytopathogical sub-model for downy mildew due to its very high pressure in 2007 and 2008. We use pluriannual data obtained by IFV on untreated plots, where severities on leaves and on grapes have been measured at weekly pace for downy mildew. We chose a parcel close to INRA Bordeaux site where the outputs of STICS-Vigne model were obtained. We searched for a set of parameters that minimized the RMSE[4] between observed data and simulated outputs. For simulated outputs we assumed that the incubation period equalled the latent period.

To validate this set of parameters, we need a larger set of observed data on plot – year pairs, with corresponding outputs from the STICS-Vigne model.

Using the bio-economic model

On the basis of this first calibration for downy mildew, we made simulations for a "Merlot" plot on the climatic data of INRA-Bordeaux "La Ferrade", from 1988 to 2008.

We compared five treatment strategies: 3 systematic strategies with treatments beginning when the vine had 6 leaves until ripening and a treatment frequency of 14, 21 and 28 days; the "Midium" strategy; and as a reference an untreated strategy.

We obtained, for these five strategies, charts for the simulated damages on leaves and grapes during these 20 years (Figure 2 & 3).

With *ad hoc* economical data (maximum yield of the AOC, sale price of grapes and costs for treatments and observations), we can also present, with box-plots, simulated statistics for the economical results and the number of treatments (Figure 4).

Moreover, we calculated the level of a "tax on agrochemicals products" that equal the economical margin for the "14 days" systematic strategy and the "Mildium" strategy. This level depends on the sale price of grapes, the treatment input cost and the total treatment and observation costs. The multiplicative factor on the treatment input cost is between 3 to 7.

On the same basis, we calculated the increase in grape price that equal the average margins for these two strategies; that is between 4 to 10%.

Perspectives

We must validate this first calibration for downy mildew on a larger set of plot – year pairs.

The same approach will be used to calibrate and validate our model for powdery mildew.

Furthermore, to take into account the joint management of these two pathogens in the "Mildium" strategy, we need to manage these two pathogens with the same model.

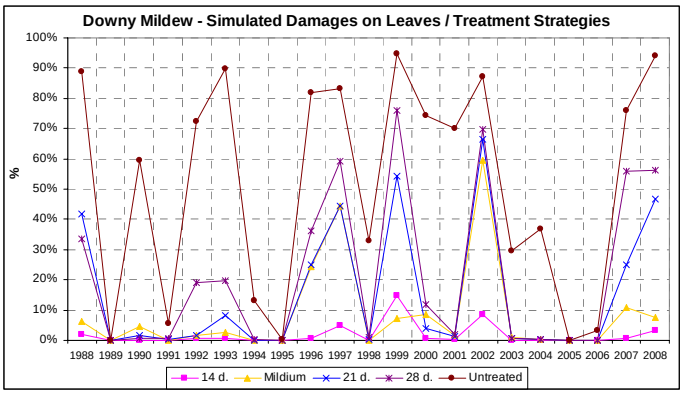

Figure 2: Simulated Damages on Leaves

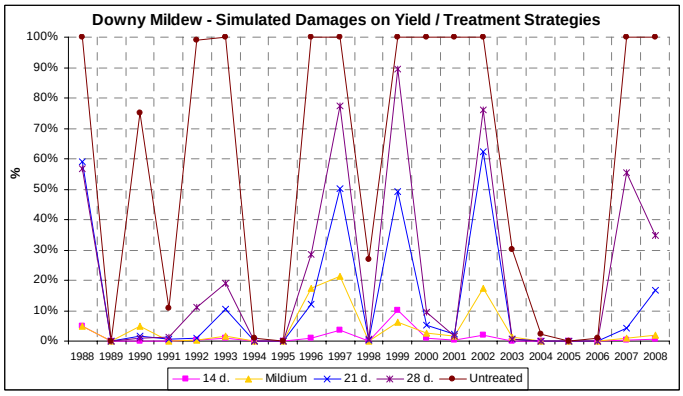

Figure 3: Simulated Damages on Yield

[3] See Cartolaro Philippe & Naud Olivier contributions on this workshop.

[4] RMSE : Root mean squared error

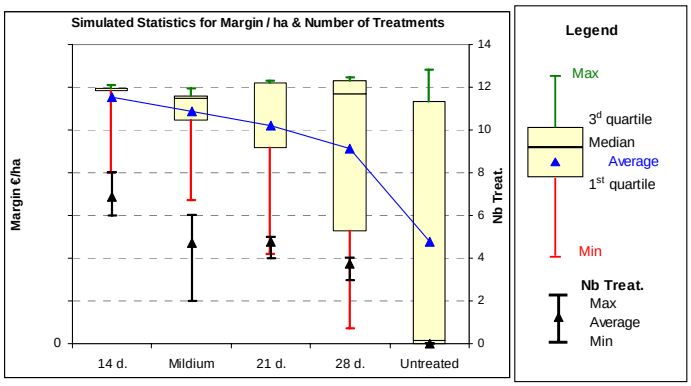

Figure 4: Simulated Statistics for Margin €/ha and number of treatments

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EPIcure, a geographic information decision support system risk assessment of downy and powdery mildew epidemics in Bordeaux vineyards

M. Raynal, C. Debord, S. Guittard, M. Vergnes

Institut Français de la Vigne et du vin (IFV) 39, rue Michel Montaigne, 33290 Blanquefort, France

Since 2007, an experimental partnership is being developed between Meteo France and French Vine and Wine Institute (IFV) to assess the impact of fine scale meteorological data on the quality of downy and powdery mildew risk of epidemics in the Bordeaux vineyard region.

Three kinds of data are tested:
- Antilope: rainfall data are obtained by the combination of radar information to locate the rainfall areas and gauges deployed to evaluate the quantification of rain; these data are delivered at 1km² scale.
- Aroma: a temperature quantification given by modeling approaches at a 2x2km²resolution. Aroma replaces Safran which provided temperature quantification at a 6x8 km² resolution.
- Weather forecasts provide an 8-day forecast of conditions. The first three days of prediction is evaluated daily by a specialist; from day+3 to day+8 the statistical prediction is given by models. Three types of scenarios - most probable, low and high - are established for six areas of the Bordeaux vineyard region and are used daily to run epidemic models.

These data are used to run the "Potential System" models (PSM), software developed by S. Strizyk from the SESMA company, for downy and powdery mildews and other pathogens.
The risk evaluation is progressively established on almost 6000 points of calculation instead of the 50 weather stations formerly used to represent the 120,000 ha in the Bordeaux vineyard region.
Whatever the measure, radar or weather stations, the weather or epidemic results are represented by means of geographical information system (GIS) named EPIcure (http://www.vignevin-epicure.com) as showed in Figure 1.
Observations made in 2007 confirmed the downy mildew PSM ability to discriminate high variation in the destruction rate due to weather conditions at the fine scale of a few kilometers.
On the 24th of May, a violent hailstorm produced 107 mm of rain on a 2 to 3 km² cluster on the Fougeyrolles vineyard (dpt 24). This event was not registered by the weather station network which indicated at the same time a maximal rainfall of only 8 mm located on the Medoc vineyard. A few weeks later, at the beginning of July, the downy PSM model registered at this location an estimation of 60% downy mildew, while it also registered just 2% at a 3 kilometers distance.
As for weather stations, the event was not confirmed because none of the almost 60 untreated plots used to monitoring epidemic development confirmed the observation at Fougeyrolles vineyard.

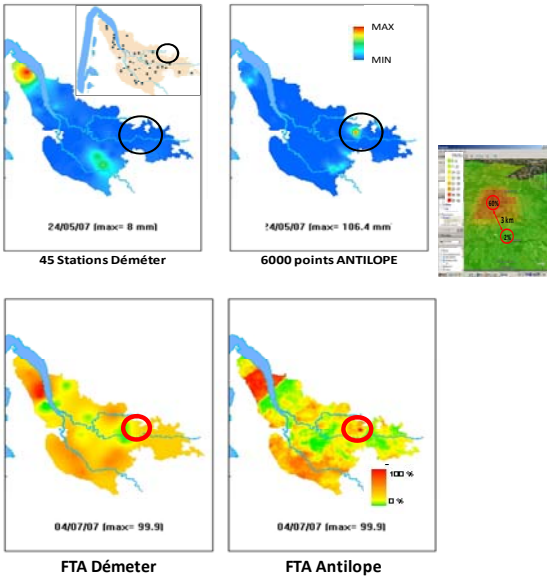

Figure 1: Spatial representation of the 24 May 2007 thunderstorm on the theoretical incidence of attack by downy mildew on 4 July in the Bordeaux vineyard region using the program Downy Mildew PSM.

IFV decided to develop a new monitoring protocol, based on a sample survey method, in order to evaluate small areas of attack at the fine scale of a few hundred meters. We tested this method on hailstorms which occurred during the spring 2009.

Material and methods

Radar Signature. The radar hail signature is given by the Meteo France Melodi S band radar of Merignac (dpt 33). A threshold sensitivity study of the radar echo as been carried out by Meteo France giving the three different levels of hail : 0, no hail ; 1 possible hail; 2 : probable hail.

Biological assessment. A notation scale as been established to assess the damage observed on the vineyard:
- 0 no damage;
- 1 few impacts;
- 2 and 3 regular and numerous impacts;
- 4 very numerous impacts; and;
- 5 very strong damage with very numerous shoots broken.

Sample survey. Hailstorms occurred on 9-13 May 2009. A sample survey was done on 27-29 May in the Bordeaux vineyard region and 15 June in the southern Cognac vineyard, close to Bordeaux. Two hundred thirty-two observations were made in the vineyard region, crossing the hailstorms supposed passage given by the radar footprint.

Web sample survey. At the same time, IFV developed a web platform (http://www.vignevin-epicure.com/grele/grele.php) which has been largely distributed to all partners in the region. Using the same scale of assessment, the observer can easily provide information on vineyard damage: the platform is connected to GoogleMap cartography system which, by means of satellite view, allows the partner to locate very precisely the parcels assessed, as shown in Figure 2. Partners have made 126 assessments using the web platform during June and the beginning of July.

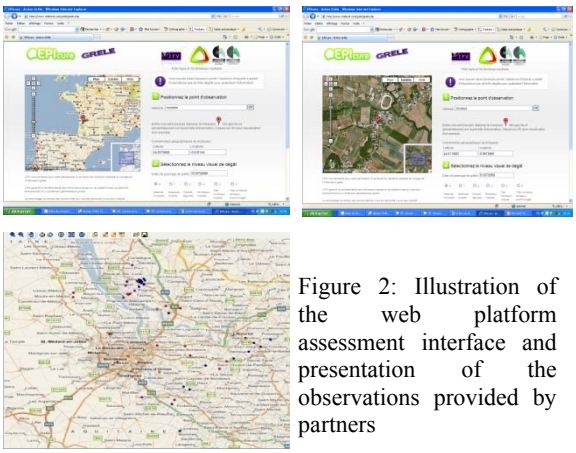

Figure 2: Illustration of the web platform assessment interface and presentation of the observations provided by partners

The radar footprint detection analysis is based on contingency tables, established for the different possible thresholds, and usually used for the receiving operating curves (ROC) methods. For each threshold defined, a status (hail predicted, hail observed) is determined and this allows evaluation of different ratios of performance by the system. Incorrect and correct detection are the two main indicators used in this study.

Results

A summary of the performance of different paired situations are presented in Table 1. Bold type figures show the best performing pairings for incorrect and correct detection.

The highest rate of correct alerts (70-775%) was for the pairing of O1/R1 which stands for few impacts / possible hail. The lowest incorrect detection alert (15-20%) was for the O1/R2 pairing, meaning few impacts observed and probable hail. Therefore, the results tend to show that the hail detection signal is not quantitative: no correlation can be done between the level of damages observed, from O1 to O5, and the hail detection possible or probable.

In a second analysis, the full data set was separated between data observed by the IFV technicians and those collected on the Web. The performance of the two new data setswas similar, whatever is the origin of the data. This shows that the experimental web platform can be effective through a web observation participative network.

The cumulative signal of hail detection level 2 (probable) analyzed between May 9th and 25th, was compared to semi quantitative observations made by IFV technicians (green bars) and received by the web platform (blue bars) (Figure3).

Table 1: Performance analysis of the most relevant pairings of observed and radar-detected hail intensities and the ability to predict the correct alert.

Threshold* Alert outcome	Data set	O1/R1	O2/R1	O1/R2	O2/R2
Incorrect	Full	**0.22**	0.54	**0.15**	0.45
Correct		**0.73**	0.52	0.61	**0.63**
Incorrect	IFV	**0.21**	0.68	**0.13**	0.58
Correct		**0.71**	0.44	0.59	**0.65**
Incorrect	Web	**0.23**	0.32	**0.17**	0.26
Correct		**0.76**	0.68	**0.64**	0.61

*O1=few impacts observed; O2=regular impacts observed; R1=hail detection possible; R2=hail detection probable.

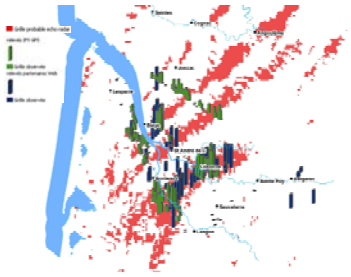

Figure 3: Illustration of the probable hailed areas detected by the Meteo France radar (red) compared to the observations made by IFV technicians (green) or received on the web (blue).

This confirms the good global correlation between the detection signal and damages observed. It also shows that the web observation network does not produce false positives (reporting hail when none occurs). This type of negative information really is almost as important as a positive declaration and should be recognized as an important methods that will not over score the phenomenon observed.

The geo-statistical descriptive analysis, when applied to a particular part of the vineyard, also demonstrated the superior ability of partners to provide high-resolution information. A wine grower from Blayais (North East part of Bordeaux vineyard region) made numerous observations on his parcels: over 22 points where monitored in a 0.49 km^2 area, whereas radar would provide a single information point for that same area. This example shows the power offered by a web observation participative network as constituted in this experiment.

Conclusion

The severe hailstorms of Spring 2009 offered us the opportunity to test in the vineyard the fine-scale meteorological tools that we conceptualized after hail storms in 2007. Since 2007, the constant and uniformly high downy mildew pressure did not allow us to drive this type of monitoring in the vineyard. Meteo France's new hail detection radar signal allowed us to perform this sample survey relevant experimentation.

First, radar allowed determination for the hailed status of a parcel with an almost 75% accuracy. However, it was unable to define correctly the damage observed by the monitoring performed by our technicians.

The web platform built to encourage contributed observations of the occurrence of hail as well as damage assessmentss by partners and wine growers was very successful. The platform has been well accepted by people and more than 100 observations have been acquired. Moreover, the quality of the data collected seems to be equal to that acquired by technicians.

Perspectives

Encouraged by these good results, we now plan to develop this web participative platform to acquire data on new subjects such as detection of first oil spots. This powerful source of information will offer a good opportunity to help us to validate the fine scale disease modelling approach.

IFV is operating a new Xray radar developed by Novimet society in the Bordeaux vineyard region. This radar should be installed in June 2010. It will permit a precise real time quantification of the rainfall (with no gauge calibration) on a 1km² resolution within a 60 km radius, which amounts to almost 11300 gauges.

Moreover, the Novimet radar is announced to be able to give a good quantification of the rainfall at a 200 to 300 m resolution (4 to 9 ha) within a 30 km radius from its settle point. IFV will develop an experimental program with Agriscope, aproducer of weather station networks, in order to evaluate climatic conditions at the sub kilometer scale. The objective evaluate if local weather variations can explain different disease trends observed in the vineyard at that same scale, and to see if the PS models are precise enough to assimilate these variations. If possible, first results will be presented during the Downy and Powdery Mildews workshop.

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This experimental program is being possible thanks to:

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McLaren Vale CropWatch: A Case study of the practical application of Downy Mildew modeling for the McLaren Vale grape growing region of South Australia.

J. M. Armstrong[a], T. Wicks[b]

[a]Integrated Viticultural Solutions, PO Box 1924, McLaren Flat. SA. Australia; [b]South Australian Research and Development Institute, GPO Box 397, Adelaide. SA. Australia 5001

McLaren Vale CropWatch was established under the umbrella of CropWatch SA® in 2003 for the McLaren Vale region, a premium grape growing region located 34 km from Adelaide. Its original aim was to reduce the reliance of grape growers on routine calendar-based sprays by encouraging the use of proactive spray strategies that optimised spray timing, the most effective fungicides, and the most efficient application methods. This was done using a vineyard pest and disease management service for the management of powdery and downy mildew. The system had three components; The first, an automatic weather station (AWS) network initially established to provide microclimatic data for the decision-support software, AusVit™, to determine likelihood of downy mildew primary and secondary infections developing and calculating the date of oil spot appearance. Secondly, vineyard monitoring was conducted to confirm the presence of pests and diseases in vineyards and thirdly a bulletin style newsletter was sent to grape growers during the growing season.(Magarey, Dixon *et al.* 2006)

CropWatch McLaren Vale has continued to develop since it's inception in 2003, to reflect the needs of the McLaren Vale grape growing community, variances in environmental and climatic conditions and continually improving technology. This paper briefly outlines the changes to McLaren Vale CropWatch system and focuses on its application as a Downy Mildew management tool for the region.

Modifications to McLaren Vale CropWatch since 2003 included purchase of a new AWS network, implementation of Standard Operating Procedures for pest and disease monitoring, photographs used to illustrate potential problems, a weekly summary of potential grape vine issues, provided and an SMS system to alert growers of primary downy mildew infection conditions. The majority of growers now receive bulletins by email which includes in addition to pest and disease, information on a broader range of subjects relating to grape growing specific to the region such as, canopy management, irrigation and weather summaries.

Downy mildew infections do not occur in McLaren Vale each season, and results in growers adopting 3 different approaches to management of primary downy mildew infections. These are:

- regular applications of protectant fungicides,
- application of protective fungicides when weather conditions conducive to a downy mildew infection are forecast and;
- application of eradicant fungicides post primary downy mildew infection prior to secondary infection.

The 'D-Model' was developed for Australia as a disease simulator for the occurrence of primary and secondary downy mildew events. At present AusVit™ is the only commercially available software containing the D-Model, with changes in computer languages direct importation of weather data into AusVit™ soft ware is no longer possible. A manual guideline has been established to model downy mildew.

The following changes since the inception of McLaren Vale CropWatch in 2003 have increased grape growers reliance on the accuracy of downy mildew modeling in the region:

1. Change in agrochemicals suitable to eradicate downy mildew post primary infection period on grapes destined for export wines.
2. Perceived change in weather patterns: an increase in number of days prior to fruit set where minimum daily temperatures are above 8oC with greater than 10mm of rain.
3. Increased in the number of grape growers implementing organic and biodynamic practices in vineyards.
4. The perception that downy mildew is not an economic threat to grapes, resulting in fewer growers applying protectant fungicides.
5. Current economic conditions have resulted in grape growers reducing expenditure.

In recent years the development of downy mildew in vineyards sited with AWS or sites close by has not necessarily concurred with the downy mildew predictions from the model used by McLaren Vale CropWatch. This paper also reviews a number of weather data sets, in relation to field observations and discusses possible reasons for the anomalies between field observations and the model.

This raises a number of questions relating to model components and interpretation of model outputs for use across an extended geographical region and includes:

- What soil moisture levels are required to initiate oospore germination?
- What tolerance should downy mildew models allow to account for microclimatic variation?
- What density of AWS is needed to predict primary downy mildew infections for a district?
- How should AWS indicator grapevine blocks be managed?
- What is the most appropriate spray regime for various approaches to vineyard management (conventional, organic or bio dynamic)?
- Are the original aims of CropWatch still appropriate in the current economic climate?

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Downy and powdery mildew models integtrated in the forecasting system "VitiMeteo"

G. Bleyer[a], H-H. Kassemeyer[a], O. Viret[b], P-H. Dubuis[b],A-L. Fabre[b], B. Bloesch[b], W. Siegfried[c], A. Naef[c], M. Huber[c], R. Krause[d]

[a]Staatliches Weinbauinstitut Freiburg, Merzhauserstr. 119, D-79100 Freiburg ; [b]Agroscope RAC Changins, Route de Duillier, CP 254 CH-1260 Nyon 1; [c]Agroscope FAW Wädenswil, Postfach 185, CH-8820 Wädenswil. [d]GEOsens Ingenieurpartnerschaft, Gewerbestraße 17, D-79285 Ebringen. Germany

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The forecasting system "VitiMeteo" was developed with the objective to create modern and flexible tools for research and further development of models. Another very important purpose was the application of "VitiMeteo" in practical viticulture. "VitiMeteo" is a cooperative project between the State Institute of Viticulture and Enology, Freiburg (Germany), the Swiss Research Station Agroscope Changins-Wädenswil (Switzerland), and the company GEOsens, Ebringen (Germany).

The core of this system is found in the database "Agrometeo", where all data from different weather stations are stored. The purpose of "Agrometeo" is to integrate weather data into various other software models (Figure 1). Basically the system consists of data sources (weather data), a database, the expert models (software) and the presentations on the Internet. The data flow is organized in the following steps: Weather data is stored in a database. The expert models receive the necessary parameters from the database. The first "VitiMeteo" module to be created was "VitiMeteo Plasmopara" in the year 2002; it calculates the most important stages of the life cycle of downy mildew (*Plasmopara viticola*). The next component was "VitiMeteo Growth" which was programmed in cooperation with Hans-Reiner Schultz from the Geisenheim Research Center (Schultz 1992, 2003). More software followed, such as "VitiMeteo Oidium", "VitiMeteo DataGraph" and "VitiMeteo Insects". Walter Kast, from the State Institute for Viticulture, Oenology and Fruit Technology Weinsberg, was able to create an algorithm based on "OiDiag-2.2", which calculates the powdery mildew risk. It is also possible to calculate the dates when the spraying against powdery mildew (*Erysiphe necator [Uncinula necator]*) should begin and the time increments between each spray (Kast 2009). The algorithms are integrated in the powdery mildew risk model "VitiMeteo Oidium". Weather data is controlled and presented with "VitiMeteo Data Graph".

The expert software allows standard calculations for use in practice. They permit also the parameterization of the most important biological developmental steps like primary infection, sporulation, and secondary infection. Also, biological parameters can be calculated for freely chosen periods and different weather stations. The parameterization of the models proved to be an advantage in the development of the modelsso it is possible that the models can be continually optimised. The structures of the models allow for continuous development; for example it is possible to replace the current calculation of the primary infection with a new better one.

Validation of the model "VitiMeteo Plasmopara", by comparing data from field observations and calculated infections, usually shows a good correspondence (Bleyer *et al.* 2009, Gobbin *et al.* 2007, Viret et. al. 2007). Since 2008 VitiMeteo Plasmopara" has also been validated in northern Italy and South Tyrol by the advisory group for fruit and viticulture in South Tyrol and the advisors of the „Cantina Mezzocorona" in Trentino and Si
cily. There „VitiMeteo Plasmopara" is considered to be useful tool for decision in viticulture plant protection. Anyhow the model should be optimised mainly in the calculation of the primary infection, the intensity of the sporulation and the secondary infection.

The company Meteoblue (Basel, Switzerland) has provided weather forecasts, which have been integrated with our software modules since 2009 (Figure 1). This is the first time that a current forecast of biological processes e.g. incubation period or growth has been possible. For example, Figure 2 demonstrates the weather data, the downy mildew risk, vine growth and the forecasted weather data, infection risk and vine growth for 5 days (behind the grey area) ahead. Figure 3 shows the powdery mildew risk, vine growth and the forecasted weather data, powdery mildew risk and vine growth for 5 days (grey area). We also create pure weather graphics with "VitiMeteo DataGraph" where the forecasted weather is integrated. This information allows the advisors to give better recommendations and the vine-6growers are able to plan their applications more precisely. In case of downy mildew it is possible to decide whether a curative or protective fungicide application is necessary immediately or if it is possible to spray a protective fungicide in a few days before a predicated infection (Bleyer *et al.* 2003). Also, in case of powdery mildew it is possible to do more targeted treatments. The integration of the weather forecast is considered as a milestone in the development of the "VitiMeteo"-system. More validation of the relevant forecasted data will be done in 2010, because the company Meteoblue (www.meteoblue.com) has optimised their weather forecast in winter 2009.

With the forecasting system "VitiMeteo", actual data referring to infection risk and to other important information for plant protection, is created twice a day on the Internet for free (www.vitimeteo.de, http://www.agrometeo.ch/). The system gives information about downy mildew, powdery mildew, grape berry moth and weather data. Finding information on the Internet is often difficult, to illustrate important information on the Internet very well, the company Geosens has programmed "VitiMeteo Widget". The software called "VitiMeteo Widget" that can be downloaded for free. "VitiMeteo Widget" is a program that uses the latest graphics (Figure 4) for weather, downy mildew, powdery mildew and grape berry moth. If you have installed "VitiMeteo Widget" on the personal computer the information appears directly on your desktop. You can choose your weather station and it's possible to enter the website www.vitimeteo.de.

In contrast to other forecasting models, the three research institutions own the computer software. The development is not dependent on commercial success because the research institutes own the software modules of the "VitiMeteo system". The Research institutes task the company GEOsens with programming, in the first line for them but the Software modules may be purchased. The majority of the earnings will be used for further development of the "VitiMeteo project" while the other part will be used by GEOsens for distribution and support. The software is regularly updated. If necessary, current research results are integrated, calculations checked, presentations of the results optimised and user's wishes for improvement of the system considered. The requirements of the "VitiMeteo" forecasting system can be entered quickly, making it an extremely flexible system.

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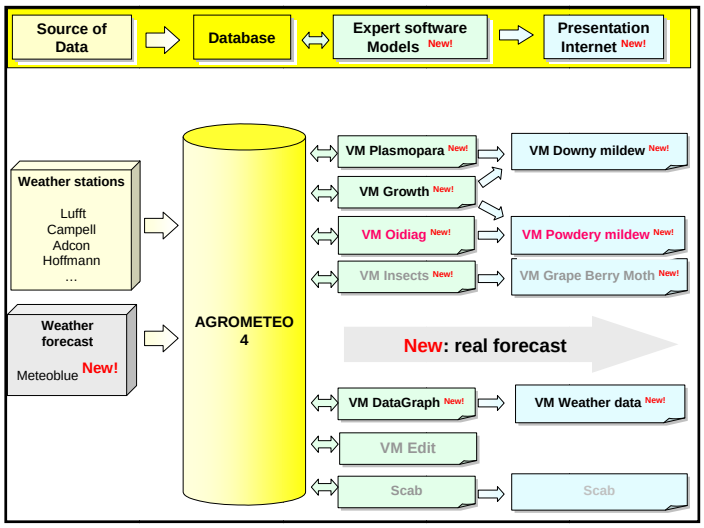

Figure 1. Structure of the forecasting system "VitiMeteo" in 2009: Overview shows how weather data is acquired, processed and subsequently offered to the user as calculated model.

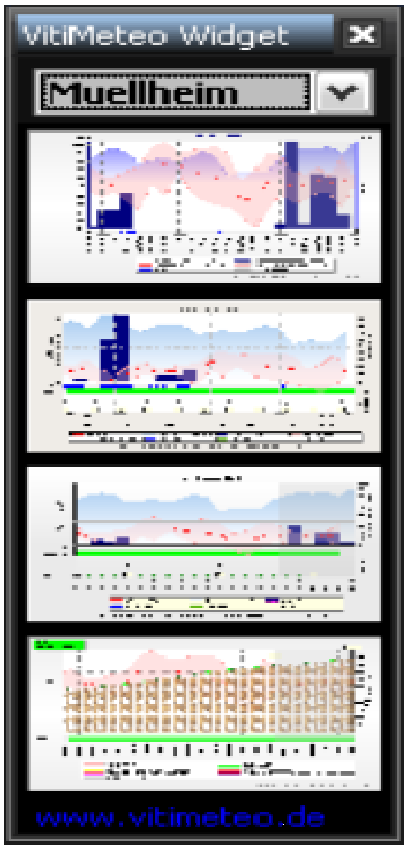

Figure 4: "VitiMeteo Widget" shows actual information at once on the desktop

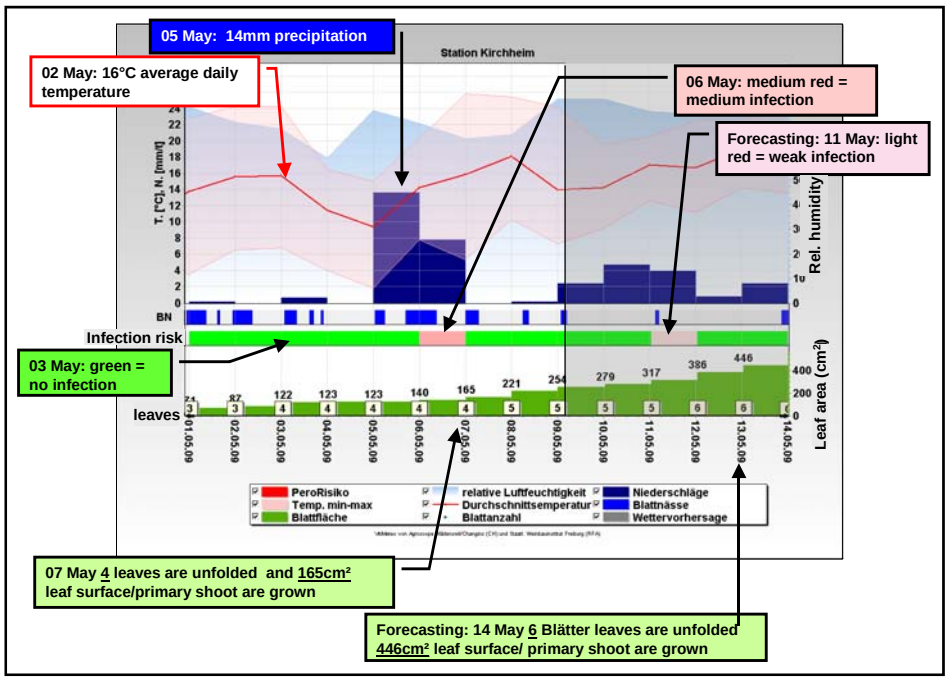

Figure 2: Downy mildew – Risk graph; weather data, infection risik and vine growth (daily) from 01 to 09 May; forecasted weather data, infection risk and vine growth for 5 days (grey area) from 09 to 14 May

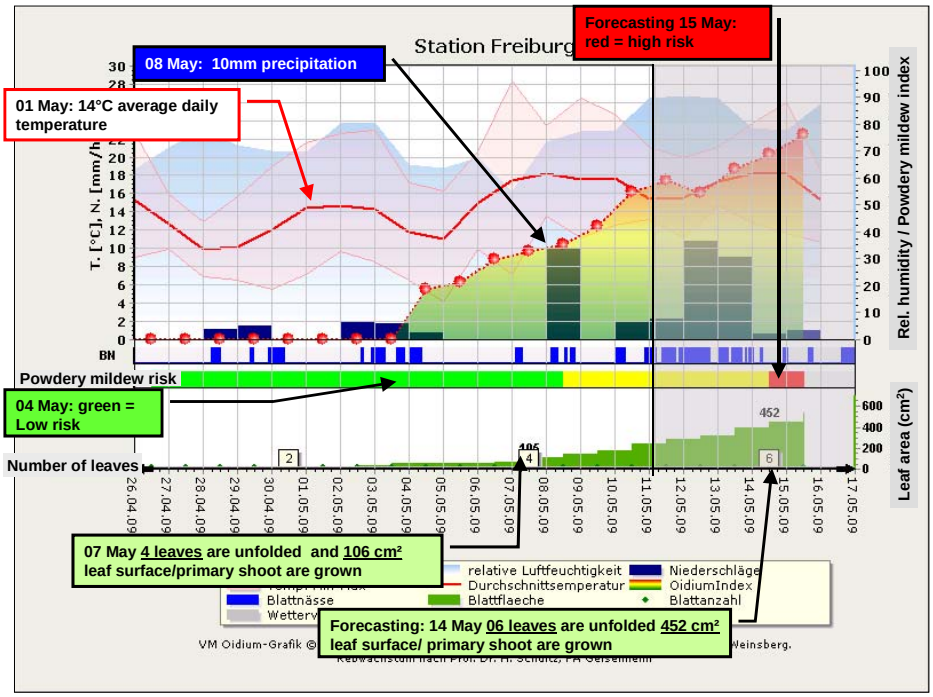

Figure 3: Powdery mildew – Risk graph; weather data, Powdery mildew risk and vine growth (daily) from 26 April to 17 May; forecasted weather data, Powdery mildew risk and vine growth for 5 days (grey area) from 11 to 16 May

The expert system OiDiag-2.2. - a useful tool for the precise scheduling of sprays against powdery mildew of vine (*Erysiphe necator*) Schwein.

W K. Kast, K. Bleyer

State Institute for Viticulture, Oenology and Fruit Technology, Traubenplatz 5, D-74189 Weinsberg, Germany

Summary
OiDiag-2.2: is a system of two tools. The first helps vine growers find the starting date for sprays against powdery mildew (*Erysiphe necator*). The second considers climatic and ontogenetic inputs to calculate an index value for the risk of powdery mildew attack. Vine growers are provided a table of relevant fungicides with values for the maximal time lag after the spray, which is completely covered by these fungicides considering these index values.

Introduction
Powdery mildew, in spite of the development of highly effective fungicides in southern Germany, is an upcoming problem because of favourable climatic change in this region (Rupp and Kast, 2009*).* In order to get the best results in the control of powdery mildew, winegrowers need to know a suitable date for the first application of fungicides and the maximal permissible time lag for the subsequent sprays. OiDiag-2.2 is a system of two separate tools that allows determining the date and the time lag for these applications. The OiDiag-2.2-system was developed to improve the OiDiag-1.0-tool (Kast, 1997). It combines knowledge about ontogenetic resistance of grapes (Stark-Urnau and Kast, 1999), the influence of climatic conditions, and long-term observations of powdery mildew development.

Material and methods
For the calculation of the date of the first application of fungicides, analyses referred to over 53 years records of temperature from the Wuerttemberg wine region and of the powdery mildew disease severity (Kast et al., 2004). Records of disease severity were taken by different individuals using a six-class system: 0 = no powdery mildew, 1 = only a few leaves with late season powdery mildew, 2 = late season symptoms in a few vineyards, 3 = late season symptoms in most vineyards on leaves and lateral grapes; 4 = a few vineyards had powdery mildew on grapes, 5 = more than 5% of the vineyards had damage on grapes. Using these data, correlations and linear regressions were calculated. Based on the regression analysis, a function was developed for a practical or relevant phenological range.
The index values for ontogenetic resistance took into account the findings of Stark-Urnau and Kast (1999) and Gadoury et al. (2003). In a first step, a fixed, weather-independent function based on the number of days after the three leaf stage was calculated for the vine regions Wuerttemberg and Baden. A new version OiDiag-2.3 will be integrated into the Vitimeteo-system. This system calculates the number of leaves based on the weather-data according to the model of Schultz (1992). Therefore, in future the ontogenetic function will fit more appropriately to other regions.
The climatic index was calculated by considering temperature, humidity, rainfall and leaf wetness. For the temperature index, the findings of Delp (1954) and Fessler and Kassemeyer (1995) were considered. For the humidity index, the findings of Delp (1954) were neglected but the humidity function of OiDiag 1.0 (Kast, 1997) was reused because this relation was mainly confirmed by Carol and

Wilcox (2003). Infections under completely dry conditions (full days with RH <65%) are of minor relevance for the

epidemic. It was assumed that the humidity and temperature both should be close to the optimum for the fungus. Therefore, the indices of humidity and temperature were combined by multiplying both factors.
No relevant published data were found for the influence of rainfall and leaf wetness. The findings of Gadoury and Pearson (1990) about ascospore release show that low rain volume in a special period may even favor the disease. As a result, a rainfall part index was developed as a combination of leaf wetness duration and amount of rainfall. The rainfall index was integrated into the formula as a reduction of the temperature-humidity combination.

Results and discussion
Date of the first spray. The severity of powdery mildew disease was correlated significantly 1) to the mean of lowest temperatures of the two preceding winters (December-February) (r=0.45**) and 2) to the disease incidence of the preceding year (r=0.35**). Based on these results, a time lag for the first spray was calculated starting at the three leaf growth stage (BBCH13).
Date of the first spray = Date of three-leaves-stage + (1,3xT-5xD + 12) days, where:

T= mean of the absolute minimum temperatures of the two preceding winters (assumption: they are always negative and lower than -5°C)

D= disease severity of the vine-site in the last year (6-class index)

When disease severity in the preceding year was extreme and during warm winter conditions, the first spray is recommended at the three leaf stage (T = -10°C, disease record D=5). If no disease was reported and low temperatures were measured during the preceding winters, the start of the sprays is recommended five weeks after the three-leaf stage, which is normally after fruit set.
Counting of spores (Flacy et al., 2007) could be an alternative to the use of this function but is much more time consuming and expensive than the use of weather data.

Maximum time lag for the subsequent sprays, the OiDiag-2.2 Index. Indexes were calculated as a base for the maximum time lag for following sprays. These index-values integrate the calculations for ontogenetic resistance, temperature, humidity, rainfall and leaf wetness.

Ontogenetic index [On]. The ontogenetic resistance of grapes was factored according to Stark-Urnau and Kast (1999). Index values were higher for the time span 10 days before and after the blossoming period and decreased after this period of extreme susceptibility (Figure 1). A function based on number of days past the three leaf stage was used, which

proved suitable for the wine regions Wuerttemberg and Baden.

ON = (D^3 x 0.0002 + (SQRT(D) x 176.6)-(LN(D) x 114,9*)-(D x 14.0)-66.0))/100)

D= number of days since 3 leaf stage

The Vitimeteo-system used the Geisenheim model of Schulz (1992) for the development of a number of leaves. A similar function depending on the number of leaves offered the chance of a function with broader adaptation to different climatic situations. It is intended to allow experienced users to set their own ON-values in a table.

Climatic index [CL]. The calculations of these indices were based on the input of (daily mean) temperature, hours with humidity between 65-80% and hours with humidity >80% per day, duration of leaf wetness (hours per day) and the amount of rainfall (mm per day). The climatic index was calculated from three indices: temperature (T), humidity (H) and rainfall and leaf wetness (R) where T= temperature part index, H= humidity part index, and R= rainfall and leaf wetness part index.

The calculated value was limited to a minimum of zero and a maximum of 1.1.

Temperature index (T). For the calculation of the temperature index, daily mean values were used. By use of multiple regressions, a function was developed that best fitted to the data of Delp (1954) and Fessler and Kassemeyer (1995).

T= (((0.11xTM)-(0.0025xTMxTM))-0.6)x1.63

TM= daily mean of temperature

Humidity index (H). For the calculation of the humidity index, the function developed for OiDiag 1.0 was used. OiDiag 1.0 weighted hours with a humidity level between 65-80% to 0.7 and more than 80% to 1.0.

H = (H80+(H65x0.7))/24

H65 = hours with humidity >65 and <80

H80 = hours with humidity >80

For values below 65%, the contribution to the OiDiag part index humidity is 0. This is used in spite of the results of Delp (1954) and Caroll and Wilcox (2003), who demonstrated that powdery mildew could infect vines even under lower humidity. The low contributions are ignored because this spreads the index values and gives a better differentiated index. The error effects of extremely high humidity near to 100% are corrected later by the rainfall and leave wetness part index.

Rainfall and leaf wetness index (R): Low rainfall events could provoke ascospore release (Gadoury and Pearson, 1990) and therefore could favour the epidemic. Rainfall events with < 2.5 mm/day were neglected in OiDiag and events of >2.5 mm/day were considered to be negative; >10 mm/day were weighted 3-fold because we assume a strong diminishing of the powdery mildew epidmemic. Leaf wetness hours are assumed to be negative for the epidemic because it may favour mildew antagonists and hyperparasites. The part index is calculated by weighting the hours of leaf wetness with the amount of rainfall where:

Table 1

Last sprayed fungicide		OiDiag index range				
		0-20	21-40	41-60	61-80	>80
Wettable Sulfur	1	14	12	8	7	6
Topas	2	14	12	9	8	7
Systane 20 EW		14	12	9	8	7
Prosper		14	12	9	8	7
Discus	3	17	15	13	10	9
Stroby		17	15	13	10	9
Vento Power		17	15	13	10	9
Universalis		17	15	13	10	9
Cabrio Top	4	23	18	15	12	11
Talendo		23	18	15	12	11
Vivando		23	18	15	12	11
Flint		23	18	15	12	11
Collis		23	18	15	12	11

if PREC <2.5mm, R1 = 0, otherwise R1= LWET x 1.5

if PREC >10 mm, R = LWET x 3, otherwise R = R1:

PREC = rainfall/day [l/m²]

LWET = Leaf-wetness [hours/day].

OiDiag 2.2 index-values. Index for a specific day. The index of a specific day was calculated by multiplication the ontogenetic index (On) with the global climatic index. Indeed, each factor can limit the upcoming infections and disease could propagate best if ontogenetic resistance is low and if optimal climatic conditions exist where:

Id=Cl x On; Cl=climatic index; and On=ontogenic index.

Sliding means. Sliding means of the daily index values for the last seven days were calculated as final results. A period of seven days seems to be the most relevant result or practical application since this is the shortest spray interval. In a period of seven days under optimal conditions, the pathogen may develop new spores. Therefore, good climatic conditions for the fungus over this 7-day period define the maximal risk in field. These final values are given out as % values for better practical handling.

Mean(($Id_{(day-6)}$, $Id_{(day-5)}$, $Id_{(day-4)}$, $Id_{(day-3)}$, $Id_{(day-2)}$, $Id_{(day-1)}$, $Id_{(actual\ day)}$)x100)

Application of index values under consideration of the active period of fungicides. The OiDiag index values were collated into five classes. (Tab 1): Fungicides were cited for the preventive period for each of these five classes and results were summarized in a table that allows winegrowers to take a reading for the next spray.

All information available, our own results and published results were used to establish a draft and the results were finally discussed with members of the fungicide-

manufacturers and trade organizations. Best information was given by the remarks about the fungicides of competitive organizations. The time lags ranged from 6 to 23 days depending on the fungicide and the index value. The range of fungicides is not fixed over time. New fungicides will be developed and the effect of some fungicides will decrease by the development of or a shifting of resistance.

Application Test in 2009. 12 wine estates in the Württemberg wine region with 43 different vineyards used the information of OiDiag for planning their sprays in 2009. Powdery mildew disease severity was generally low in 2009. In 34 of these vineyards, the first spray and the time lags were according to OiDiag or even earlier or shorter. In none of these vineyards was powdery mildew found. The required intervals were not fulfilled in seven cases. In four of these, disease symptoms on a low level were found (maximal disease severity 2.4%). One estate with four vineyards started too late and had powdery mildew disease (severity 0.5%) in a high susceptible variety Trollinger. However, the results in 2009 are less relevant because of the low disease pressure. An MS® Excel sheet with the complete OiDiag-2.1 calculations can be freely downloaded from www.lvwo.bwl.de or from www.OiDiag.de.vu.

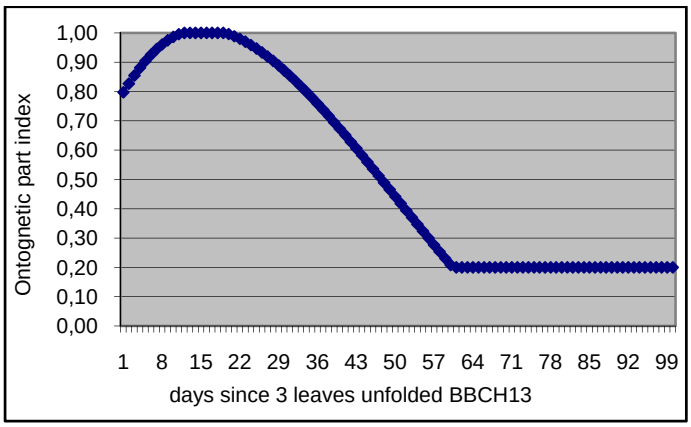

Figure 1: Ontogenetic index used for OiDiag 2.1 and 2.2 in 2009, based on number of days past the three-leaf stage.(x=days past three-leaf stage). These calculations will be replaced by the number of leaves of the temperature dependent Geisenheim model (Schultz 1992) which is already in use for Vitimeteo (OiDiag-2.3).

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Continuous research to propose solutions against powdery mildew

G. Labourdette[a], H. Lachaise[a], H. Rieck[b], D. Steiger[b]

[a] Bayer SAS, Bayer Cropscience, 14-20 Impasse P. Baizet, FR-69009 Lyon – France, [b] Bayer CropScience AG, A. Nobel Strasse 50, D-40789 Monheim am Rhein - Germany

Powdery mildew (*Erysiphe necator*) is a worldwide spread disease affecting temperate and tropical vineyards. The disease affects the plant growth from bud emergence to wood hardiness stages, reducing the yield and the quality of the final produce for wine or table grape production. The control of powdery mildew in commercial plantations is most commonly based on the use of fungicides with up to seven treatments per season. Beside sulfur, several fungicides groups with specific mode of action were developed in the last fifteen years: SBIs, QoIs, quinoleines, benzophenones, amidoxime and first generation of succinate dehydrogenase inhibitors (complex II of the respiratory chain, SDHI) .All these specific fungicides require resistance management including the limitation of number of treatments and ready-mix solutions. It is therefore crucial to have a number of highly efficient tools available. Bayer CropScience proposes continuously innovations to control powdery mildew in vineyards. Fluopyram, a fungicide from the new chemical class of pyridinyl ethyl benzamides, is specifically developed for controlling problematic diseases in a broad range of crops. It affects the fungi at all growth stages from germination to sporulation. Its biochemical mode of action has been shown to rely on the inhibition of the enzyme succinate dehydrogenase. Unlike all the other SDHI recently discovered, fluopyram presents a unique highly flexible chemical structure and a unique spectrum of activity with a lower dose rate. It provides a very good level of control of powdery mildew diseases and an excellent selectivity for the crop. Its particular behaviour in the plant allows fluopyram to protect all growth stages of the disease. When applied on the plant, fluopyram is present at the surface of the leaves and the berries. It is taken up and transported translaminarly and acropetaly thus protecting the entire plant, even during fast growth periods. Thanks to the excellent field efficacy, fluopyram and the based products provide an outstanding in season disease control resulting in better quantity and quality of the yield. Fluopyram is developed in combination with other fungicides resulting in a family of products that offers a wide spectrum of activity and robust resistance management tools. Fluopyram will be developed worldwide with globally established MRLs and import tolerances. In all, fluopyram gives to the wine grape growers and the table grape producers, an innovative solution for a more sustainable production and an easier free trade.

Key words: Complex II inhibitors, *Erysiphe necator*, fungicide, fluopyram, Grape, Powdery mildew, SDHI.

Ecology, Toxicity and Efficacy; How fungicides for downy mildew and powdery mildew in grapes have changed since 1970

HD Armstrong, RT Loveless

Bayer CropScience, 391-393 Tooronga Rd East Hawthorn Vic. 3123, Australia.

Background

- Integrated management systems have proven to be the most sustainable techniques for managing a range of commercial crop diseases and pests.
- Fungicides continue to play an important role in the management of powdery mildew and downy mildew in grapes.
- The development of new chemistry over the past 40 years has presented both opportunities and challenges.
- Increased focus on user safety, environmental impact and ecotoxicology has resulted in both regulatory and consumer demands to demonstrate 'low impact'.
- The study of base line 'impact' is a complex area, particularly when taken from controlled laboratory or modeled studies into the field situation.

Method

A timeline since 1970 of available fungicides for these diseases in grapes will be discussed.

Baseline toxicity and ecotoxicity data will be compared.

Estimates of the in field impact in a model vineyard will be made comparing various fungicide regimes across low and high pressure climates for both powdery and downy mildew.

Reference will be made to independent ratings of fungicides and the contribution of fungicide alternatives in managing these diseases.

Application of Profiler® fungicide (a.i. fosetyl-Al+ fluopicolide) in control of grapevine downy mildew in Montenegro

N. Latinovic, J. Latinovic

University of Montenegro, Biotechnical faculty, Mihaila Lalica 1, 81 000, Podgorica, Montenegro

Grapevine downy mildew is one of the most significant grapevine diseases. It is caused by the phytopathogen *Plasmopara viticola* (Berk. & Curt.). It can cause considerable damage reducing yield up to 100% in certain years. In vineyards in Montenegro, it appears every year in lower or higher intensity and fungicides are necessary for its control.

During 2009, the biological efficacy of a new product Profiler® (a.i. fosetyl-Al+fluopicolide) for Montenegro was examined in trial field at the Biotechnical faculty. The product contained the new active ingredient fluopicolide (chemical group acylpicolide) with a new mode of action. It influences spore germination, mycelium growth and sporulation of the fungus (Gouot, 2006; Toquin *et al.*, 2006).

Materials and Methods

The trial was set up in randomized block design with four replications, according to EPPO methods (EPPO, 1997). A replication comprised 5 vines of Vranac cultivar with 100 examined leaves. Seven treatments were applied as follows:

1) two days before flowering on May 15 (BBCH 57);
2) May 25; 3) June 01; 4) June 06; 5) June 18; 6) June 30; and 7) beginning of ripening on July 08 (BBCH 81).

Profiler® was applied in two doses: 2.25 kg/ha and 3.0 kg/ha. Verita® (a.i. fosetyl-Al+fenamidon) at dose 3.0 kg/ha was used as a standard. Treatments were carried out with a STHIL motorized sprayer at water consumption of 1000 l/ha.

Biological efficacy of the fungicides was checked two times: first on June 20 (BBCH 79) and second on July 14 (BBCH 83).

Clusters in the trial were harvested and weighed on September 10 in order to establish the influence of different treatments on grapevine yield. In each assessment, 100 leaves per replication were also evaluated. The percentage disease development on the leaves was rated in 12 levels according to EPPO standards scale (EPPO, 1997). Disease intensity of grape downy mildew was calculated using Towsend-Heuberger's formula (Aksoy and Serdar, 2004). The efficacy of the tested compounds was evaluated using Abbott's formula (Aksoy and Serdar, 2004). Data were analysed separately for each trial using ANOVA and the means were separated by Duncan's multiple range test.

Results

The first symptom of downy mildew was noticed on May 31 but only on one leaf in the control treatment. From June 15, there were more symptoms on leaves in control. At the end of June, symptoms also started to appear on grapes in the untreated control while in treated plots, there were only a few symptoms on grapes treated with the fungicide Verita®.

During the vegetation period there were adequate conditions for development of downy mildew. From May 26 to May 31, there were three rain days (total precipitation 26 mm). In June, there were two rain periods. The first was with two rain days only but with precipitation of 84 mm, while in the second, there were many rainy days with total precipitation of 107 mm. In July there were only three consequent rain days with total precipitation of 17.2 mm. Average daily temperatures were 22.5°C in May, 21.8°C in June and 27,3°C in July.

In the first assessment made on June 20, the treatment with Profiler® expressed 100% efficacy in both applied doses (2.25 kg/ha and 3.0 kg/ha), while the treatment with Verita® showed efficacy of 98.3% (Tab. 1). There was no difference in disease intensity between these three treatments while there was significant difference between them and the untreated control.

Table 1: First assessment of the efficacy of seven applications of two fungicides against downy mildew on *cv*. Vranac grapevines, Podgorica, Montenegro, June 20, 2009.

Treatment	Rate (kg/ha)	Disease severity (%)	Efficacy (%)
Profiler®	2.25	0.0a	100.0
Profiler®	3.0	0.0a	100.0
Verita®	3.0	0.4a	98.3
Untreated	-	20.8b	-

Letters show significance differences ($P<0.05$), Duncan's multiple range test.

Based on the second assessment conducted on July 14, it was easy to establish an increase of disease intensity in control: it increased from 20.8% in the first assessment to 43.6% in second (Table 2).

Table 2: Second assessment of the efficacy of seven applications of two fungicides against downy mildew on *cv.* Vranac grapevines, Podgorica, Montenegro, July 14, 2009.

Treatment	Rate (kg/ha)	Disease severity (%)	Efficacy (%)
Profiler®	2.25	0.8a	98.2
Profiler®	3.0	0.3a	99.3
Verita®	3.0	8.0b	81.4
Untreated	-	43.6c	-

Letters show significance differences (P<0.05), Duncan's multiple range test.

Fungicide treatments containing Profiler® remained extremely efficient with efficacy of 98,2% if applied at the lower dose and 99,3% when applied at the higher dose. In this evaluation, disease intensity on leaves treated with Verita® was significantly higher than with Profiler® at both rates. All treatments showed significant difference compared with control.

These data are quite similar to the results achieved in two years examination of fungicide Profiler® in Serbia where efficacy ranged from 96.1 to 99.7% (Rekanović *et al.*, 2008).

The assessment of average grape yield per vine at harvest showed (Tab. 3) that, for each treatment, the greatest yield was achieved in treatments with Profiler® applied at both the lower and higher doses (4.7 and 4.83 kg/vine respectively). The Duncan test showed no significant difference between them. Significantly lower yield was measured on vines treated with Verita® (3.58 kg/ vine) while the lowest yield was in untreated control plots with 1,83 kg/vine, showing significant difference compared with all other treatments in the trial.

Table 3: Yield of grapevines after seven applications of two fungicides against downy mildew on grapevine *cv.* Vranac. Podgorica, Montenegro. September 2009.

Treatment	Rate (kg/ha)	Yield kg/ vine stock
Profiler®	2.25	4.70a
Profiler®	3.0	4.83a
Verita®	3.0	3.58b
Untreated	-	1.83c

Letters show significance differences (P<0.05), Duncan's multiple range test.

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Comparison of fosetyl-Al and another phosphonate on plant Downy mildew protection and on *Arabidopsis thaliana* Gene Expression

M-P. Latorse, L. Mauprivez, C. Sirven, P. Gautier, R. Beffa

Research Center La Dargoire,Bayer Cropscience, BP9163, 69263 Lyon Cedex09, France,
E-mail: marie-pascale.latorse@bayercropscience.com

Fosetyl-Al is a well-known anti- oomycetes fungicide of the chemical family of metallic mono-ethyl phosphonates, registered in the 1970's. Its antifungal effect could be explained for a part by a direct effect on the inhibition of the growth of oomycete mycelium (*Phytophthora sp*) by competing for the assimilation of phosphates. In addition, its efficacy could be increased by an indirect action, i.e. the stimulation of the natural plant defences. The biochemical mechanisms involved in this indirect efficacy of fosetyl-Al is not yet fully understood.

To face generic compounds emergency and competitors as natural plant defences stimulators, interest for fosetyl-Al mode of action comprehension has been encouraged with the development of new technologies available since recent years (*e.g*. genomic tools on model plant such as *Arabidopsis thaliana*).

The aim of this project was to study in *A. thaliana* the level of transcripts (mRNA) by using microarrays (Affymetrix chips) after preventive application of the products, followed or not by an infection with the oomycete *Hyaloperonospora parasitica*.

Correlated to a protection against downy mildew, data of transcriptome analysis allowed to illustrate the priming effect of phosphonate based products. In addition gene expression profiles obtained after treatments with fosetyl-Al and another phosphonate on infected plants showed overlaps but also differences. This effect of fosetyl-Al will be discussed in relation to its better ability to protect the plants against *H. parasitica*.

Production and eradication of overwintering inoculum of *Erysiphe necator* in Michigan vineyards

L. L. Avila[a], K. L. Powers[b], N. L. Rothwell[b], S. Nagendran[a], A. M. C. Schilder[a]

[a]Department of Plant Pathology, Michigan State University, East Lansing, Michigan, USA.
[b]Northwest Michigan Horticultural Research Station, Michigan State University, Traverse City, Michigan, USA.

Grape powdery mildew is caused by the ascomycete fungus *Erysiphe necator* Schw. During the growing season, this biotrophic pathogen asexually reproduces by producing numerous chains of airborne conidia. At the end of the growing season and after mating with a compatible partner, a resistant ascocarp known as a chasmothecium (also called cleistothecium) is formed. This structure allows *E. necator* to overcome environmentally harsh conditions and ensures the viability of primary inoculum following winter (Pearson and Gadoury, 1987; Cortesi et al., 1997).

Since *E. necator* is a heterothallic fungus, initiation of chasmothecia depends on the availability of compatible mating types. Ascocarp abundance is positively correlated with a high disease incidence in any given vineyard (Gadoury and Pearson, 1991). Likewise, chasmothecium development has been studied under laboratory conditions to determine the effects of environmental factors and host biology (Gadoury and Pearson, 1988). Those studies demonstrated that temperatures below 10ºC hinder ascocarp maturation, and below 8º, ascocarp formation is halted altogether. Host susceptibility also influences the time required for ascocarop maturation. The objective of this study was to study the timing of chasmothecium production and to evaluate the ability of fungicides to eradicate existing colonies and reduce cleistothecium formation.

Using two methods, we assessed chasmothecium production on leaves of *Vitis* interspecific hybrid 'Chardonel' and *V. vinifera* L. 'Pinot Noir' in Clarksville (central Michigan) and Traverse City (northwest Michigan, USA), respectively, during 2008 and 2009. An 18-cm-diameter collection funnel connected to a 3-L plastic bottle was placed below the canopy of a vine untreated with fungicides during the growing season. Bottles were covered with aluminum foil and contained iodine to prevent sample deterioration. Four replicates were used per site. Rainwater samples were collected weekly, filtered through Whatman No.1 filter paper, and chasmothecia was counted using a dissecting microscope. In addition, 20 leaf samples were collected weekly from four unsprayed locations in each vineyard to study chasmothecium development on the leaves. Chasmothecia were counted in five 1-cm^2 areas on both surfaces of the leaves, and considered immature if they were yellow or light-brown and mature if they were dark-brown. Campbell weather stations of the Michigan Automated Weather Network were used to monitor environmental conditions at both sites. In 2009, we sampled two different locations in Northwest Michigan: an unsprayed row of mixed varieties in Northwest Michigan Horticultural Research Station, Michigan State University (MSU), Traverse City, MI. and a 'Marechal Foch' vineyard in Suttons Bay, MI, USA. Ten leaves were randomly collected weekly for quantification of chasmothecia in these vineyards.

To evaluate the ability of fungicides to eradicate existing powdery mildew colonies and reduce chasmothecium formation, the following products were applied to mature 'Pinot noir' vines in Traverse City, MI: JMS Stylet Oil (paraffinic oil), Kaligreen (potassium bicarbonate), Oxidate (hydrogen dioxide), Prev-Am (sodium tetraborohydrate decahydrate), C+G (organic acids), Cuprofix (basic copper sulfate), Sulforix (calcium polysulfide), and Elite (tebuconazole). On 13 September 2007, treatments were applied at recommended rates to single-vine plots with a backpack sprayer. About 20% of the leaf area on average was covered with powdery mildew at the time of fungicide application. Plots were replicated five times in a randomized complete block design. Spray volume was equivalent to 935 L/ha. Unsprayed vines served as controls. Disease severity was assessed immediately before and 1 and 2 weeks after application on 20 randomly selected leaves per plot. Chasmothecia were counted on 10 randomly selected leaves per plot on 17 October 2007.

Chasmothecia in rainwater traps. Rainwater samples were collected from 19 August to 9 October in 2008 and from 21 August to 1 October in 2009. In 2008, a peak in the number of chasmothecia trapped in mid-September at both sites was correlated with the highest precipitation level observed during the sampling period (Fig. 1). The average peak chasmothecium counts were 489 in Clarksville and 4907 in Traverse City. The higher numbers at the latter site was likely due to higher disease pressure in cv. Pinot Noir. Also the weather in the northern part of Michigan is generally drier and more favorable for powdery mildew development and chasmothecium formation. In 2009, the highest number of chasmothecia was observed by mid-September after 20 days without precipitation (Clarksville, Figure 1C). No chasmothecia were collected during dry periods since no rainwater was collected in the traps. Low chasmothecium numbers in October may have been due to lower temperatures and precipitation levels.

Chasmothecia on leaves. Leaves were randomly collected from cv. Chardonel vines from 4 September to 2 October in 2008 and from 4 September to 1 October in 2009. In Traverse City, leaves were collected from 24 August to 15 October in 2009 from two different locations. Chasmothecium counts on leaves were variable, with reductions apparent after rainfall events in 2008, although this was not apparent in 2009 (Fig. 2). In general, fewer chasmothecia were produced in 2009 than in 2008, which was likely due to late disease development and overall lower disease pressure due to the cool growing season that year. Not surprisingly, the proportion of mature vs. immature chasmothecia increased as time

progressed, although even at the end as freezing temperatures lead to leaf drop, there were a fair number of immature chasmothecia left. Each year, we observed much higher numbers of chasmothecia on the abaxial than on the adaxial surfaces of leaves, presumably because upper leaf surfaces are more exposed to rain events. Chasmothecia produced on the undersides of leaves are less likely to be washed off with rainwater. They may therefore fall to the ground with senescent leaves. Gadoury and Pearson (1988) showed that chasmothecia that remained attached to leaf tissues had not completed the maturation process, hence they were unable to survive the winter in New York. However, in other locations (Australia, Italy, and Washington), chasmothecia seem capable of surviving on senescent leaves on the soil surface (Grove, 2004). Further studies are needed to investigate the viability of leaf-borne ascocarps and their epidemiological significance in Michigan.

Eradicative action of fungicides. After 1 week, JMS Stylet Oil had reduced powdery mildew severity the most compared to the untreated control (by 75%), followed by C+G (59%), Sulforix (53%) and Kaligreen (39%). The other fungicides were less effective. After 2 weeks, disease severity in these treatments was reduced by 88%, 82%, 73%, and 79%, respectively, compared to the untreated control. The total number of mature cleistothecia was reduced most by Sulforix (82%), followed by Elite (78%), Kaligreen (70%), and C+G (66%). The results indicate that a late-season fungicide applications can reduce foliar powdery mildew severity as well overwintering inoculum.

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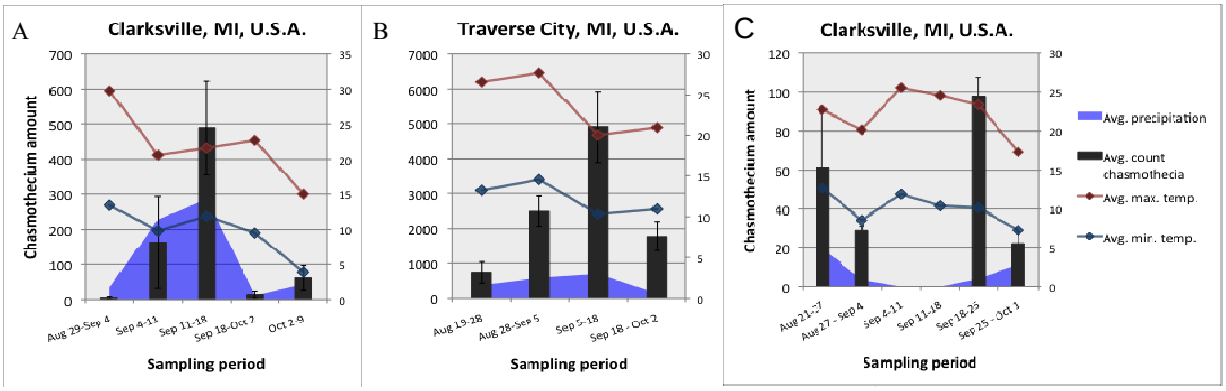

Figure 1: Average number of chasmothecia collected in rainwater samples in grapes: A) cv. Chardonel in Clarksville, MI, 2008; B) cv. Pinot Noir in Traverse City, MI, in 2008, and C) cv. Chardonel in Clarksville, MI, in 2009. Bars indicate standard errors of the mean (n=4). Precipitation (mm) and maximum and minimum temperatures (°C) are shown as an average over each collection period.

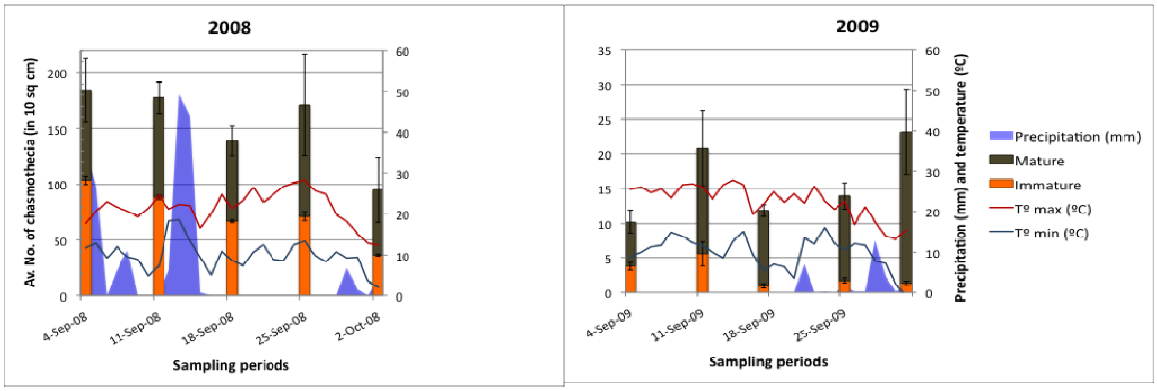

Figure 2: Chasmothecium development on cv. Chardonel leaves in Clarksville, MI, U.S.A. in 2008 and 2009. Bars indicate standard error of the mean (n=4). Precipitation (mm) and maximum and minimum temperatures (°C) are also shown.

Control of foliar diseases in viticulture using milk: understanding mechanisms

D. Godfrey[a], TJ. Wicks[b], PR. Grbin[a], DK. Taylor[a], D Bruer[c], R Crittenden, ES. Scott[a]

[a] School of Agriculture, Food and Wine, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia; [b] South Australian Research and Development Institute, GPO Box 397, SA 5001, Australia; [c] Temple Bruer Wines, RSD 226, Strathalbyn, SA 5255, Australia; sixth author: MG Nutritionals, 140 Dawson St, Brunswick, VIC 3056, Australia. e-mail: dale.godfrey@adelaide.edu.au

Grapevine powdery mildew, caused by *Erysiphe necator* (syn. *Uncinula necator*), is the most economically important fungal disease of grapevine in Australia. It is estimated to cost the Australian grape and wine industry at least AUD$30 million per year in loss of yield and quality, and costs associated with disease management (Wicks et al., 1997). While the control of powdery mildew is predominantly based on the use of sulfur and synthetic fungicides, the development of fungicide-resistance (Savocchia et al. 2004) and the demand for residue-free produce are considerable incentives for the development of alternatives. Furthermore, sulfur is known to be detrimental to beneficial mites and insects, including natural antagonists of *E. necator* (Calvert et al., 1974) and can be toxic to agricultural workers (Robinson et al., 1993).

In a study of alternatives to sulfur for control of grapevine powdery mildew, Crisp *et al.* (Crisp et al., 2006) demonstrated the potential of bovine milk to reduce disease severity in a commercial vineyard in South Australia (SA). The principal constituents of bovine milk are water (88.3%), lactose (4.6%), fat (3.2%) and proteins (3.2%) (Jenkins et al., 2006). In addition to providing a complete diet for infant mammals, milk contains biologically active components to protect against infection. However, the activity of milk components against plant pathogens is not well understood. A review of the literature has identified a number of candidates, including the glycoprotein lactoferrin and several free fatty acids (for review see Stelwagen et al., 2009). This research aims to identify the components of milk responsible for antifungal activity against powdery mildew, and to determine their mode of action. We report on experiments now in progress to assess milk and various components of milk for efficacy in controlling powdery mildew using *in vitro* assays and small plot field trials. The aim is to contribute to development of environmentally sustainable strategies for the management of powdery mildew.

In vitro assays for antifungal activity

Methods

Germination assays were performed by brushing conidia of *E. necator* (isolate APC1) from infected leaves of cv. Cabernet Sauvignon, maintained using a detached leaf system (Evans et al., 1996), onto glass slides coated with 2% water agar. Test materials were included in the agar (amended agar) or applied to the agar using an Atomizer reagent sprayer (Alltech Associates Inc., Belgium) and air dried for 90 min prior to inoculation. Slides were incubated at 22°C in a 16 h light, 8 h dark cycle for 24 h. Spores were stained with lactophenol trypan blue (0.1%) and germination assessed.

The protective and curative activity of test materials was further investigated by modifying the detached leaf system. For protective assays, leaves of cv. Cabernet Sauvignon were pretreated by floating (adaxial surface down) in test materials for 5 s, then air dried for 90 min prior to inoculation. For curative assays, leaves were treated 2 or 3 days post-inoculation (dpi) by floating on test materials then air drying as above. For protective and curative assays, leaves were incubated at 22°C in a 16 h light, 8 h dark cycle and disease severity was assessed as area affected by powdery mildew at 1 to 14 days post-treatment (dpt).

Materials tested included bovine full cream milk and skim milk (1 in 5, 10, 50, 100 and 500 dilution, Murray Goulburn Co-op. Co. Ltd.), Pauls Zymil lactose-free full cream milk and lactose-free skim milk (1:10 dilution, Parmalat), lactose (5 and 50 g/L, Sigma), milk protein lactoferrin (0.02, 0.2, 2 and 20 g/L, Sigma), various bovine milk fatty acids (50, 100, 250, 500 and 1000 µM, Sigma) and Protector^{hml} wetting agent (0.5%, Henry Manufacturing Limited). Treatments were compared with controls comprising leaves treated with water or sulfur (3 g/L, Garden King).

Mean percentage germination was compared using a *t*-test. Disease assessment data were subjected to analysis of variance (ANOVA) to test the hypothesis that there would be no significant difference in mean disease score among test materials and the control treatments. Least Significance Difference (LSD) was used to assess differences between means. The 5% level of significance ($P = 0.05$) was used for all experiments. All statistical analyses were performed using GENSTAT (Lawes Agricultural Trust) v. 12.

Results

Germination of *E. necator* conidia on agar sprayed with full cream milk (1 in 5 dilution) was significantly less (2.2%) than that on water-treated agar (26.2%). At 8 dpi, disease on leaves pretreated with full cream milk (0.6%, 1 in 10 dilution) and skim milk (0%, 1 in 10 dilution) was similar to the sulfur control (0%) but significantly ($P < 0.05$) less severe that the water control (76.7%). At 7 dpt the severity of powdery mildew on leaves treated 3 dpi with full cream and skim milk decreased with increasing milk concentration (Table 1), indicating that milk exhibited curative antifungal properties.

At a concentration comparable to 1 in 10 diluted milk, lactose (5 g/L) exhibited significant protective antifungal activity (65% of the leaf area affected) compared with water (97.3%). However, lactose-free skim milk (9%) and lactose-free full cream milk (2.3%) were not significantly different from skim (0%) and full cream milk (0.6%). At a concentration equivalent to that found in milk, lactoferrin did not exhibit either protective or curative antifungal activity against powdery mildew. Two fatty acids present in milk exhibited significant curative activity at concentrations equivalent to milk. At 10 dpt, only 5 and 17.5% of the leaf area was affected by powdery mildew, compared with 83.5% on the water-treated leaves. Conversely, protective activity was not observed. In another experiment, the wetting agent alone exhibited curative activity (6 dpt), with only 1.7% of the leaf area affected, compared with 91.7% on the water-treated leaves.

Table 1 Mean severity (%) of powdery mildew (7 dpt) on detached leaves treated 3 dpi with milk at various concentrations. Means followed by the same letter are not significantly different at $P = 0.05$.

Concentration	Full cream milk	Skim milk
water	96 a	95 a
1 in 500 dilution	78.3 b	93.3 a
1 in 100 dilution	81.7 b	63.3 b
1 in 50 dilution	33.3 c	60 b
1 in 10 dilution	0 d	21.7 c

Small plot field trials

Methods

In 2009/2010 field trials were conducted at Temple Bruer Wines, an organic commercial vineyard at Langhorne Creek SA, and at Waite Campus, Urrbrae SA. The trials were designed as a randomized complete block, with eight replicates of four vines per plot at Langhorne Creek, and four replicates of three vines per plot at Urrbrae.

At Langhorne Creek, mature vines of cv. Verdelho were sprayed with wetting agent (0.5%), bovine skim milk (1:10 dilution), full cream milk (1:10 dilution) and full cream milk (1:10 dilution) plus wetting agent (0.5%). Controls comprised untreated vines and vines sprayed with sulfur (3 g/L). At Urrbrae, mature vines of cv. Chardonnay were sprayed with wetting agent (0.5%), full cream milk (1:10 dilution) and full cream milk (1:10 dilution) plus wetting agent (0.5%). Controls comprised untreated vines.

Treatments were first applied when shoots were approximately 15 cm long and continued on a 7-18 day basis, irrespective of disease, until 27th December at Langhorne Creek and 20th January at Urrbrae. Treatments were applied using a Solo 475 backpack spray with a hand-held wand. Spray volumes increased from ~500 L/ha early in the season to ~2000 L/ha at the final application. A total of 10 and 16 sprays was applied at Langhorne Creek and Urrbrae, respectively. Disease incidence was monitored weekly throughout the growing season. Disease severity on leaves and bunches was assessed weekly as area affected by powdery mildew, with the aid of standard area diagrams (Emmett and Wicks, pers com, 2009), for 40 leaves and 100 bunches selected randomly per plot. At harvest, yield was assessed for each treatment and juice samples collected for assessment of pH, titratable acidity (TA) and ºBrix. Data were analysed as above.

Results

At Langhorne Creek, powdery mildew was not detected on Verdelho vines, including untreated vines. There were no significant differences in yield between the treatments. Results for pH, TA and ºBrix will be presented. At Urrbrae, powdery mildew was first detected on leaves of untreated Chardonnay vines in mid-November. Milk (with or without wetting agent) consistently reduced mean disease severity on leaves and berries compared with the untreated control. At harvest, the severity of powdery mildew on bunches of milk treated-vines (1.5%) was significantly less ($P < 0.05$) than that on vines treated with wetting agent (5.4%) or left untreated (7.3%). The severity of powdery mildew on vines treated with milk plus wetting agent (1.6%) was not significantly different from that of milk-treated vines. No significant differences were detected in yield between treatments. Results for pH, TA and ºBrix of juice will be presented.

Summary

Milk (full cream and skim milk) exhibited both protective and curative properties against powdery mildew *in vitro*. The curative properties appeared to depend on concentration. Two milk fatty acids exhibited curative, but not protective, activity *in vitro*. Materials shown to reduce the severity of powdery mildew *in vitro* will be evaluated further in glasshouse and small plot vineyard trials to assess efficacy in a commercial environment.

Regular application of milk reduced the severity of powdery mildew in the vineyard at Urrbrae. The addition of a wetting agent did not influence efficacy. The test materials had no obvious effect on yield, irrespective of the presence (Urrbrae) or absence (Langhorne Creek) of powdery mildew in the trial sites.

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Is the biocontrol efficacy of *Ampelomyces quisqualis* against powdery mildew related to the aggressiveness of the strain?

D. Angeli[a], E. Pellegrini[a], S. Micheli[a], M. Maurhofer[b], C. Gessler[b], I. Pertot[a]

[a]IASMA Research and Innovation Centre, Fondazione Edmund Mach (FEM), 38010 S. Michele all'Adige (TN), Italy, E-mail: *dario.angeli@iasma.it*; [b]Institute of Integrative biology, Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland.

Powdery mildews (PMs) are some of the most important plant diseases worldwide. Fungi causing PMs are obligate biotrophic parasites belonging to *Erysiphaceae.* Important crops including wheat, barley, grapevines, apple and a number of vegetables and ornamentals are the major hosts of PM fungi. PMs are frequently parasitized under field and natural conditions by pycnidial fungi of the genus *Ampelomyces* (Kiss, 1997a).

Ampelomyces quisqualis is the most studied biocontrol agent of powdery mildews (Jarwis and Slingsby, 1977; Sundheim and Tronsmo, 1988; Sztejnberg *et al.*, 1989; Falk *et al.*, 1995). *Ampelomyces quisqualis* has been found on more than 64 species of PM on 256 species of plants (Kiss, 1997b; Kiss, 2003; Kiss *et al.*, 2004) and a strain isolated in Israel has been commercialized in several countries under the trade name "AQ10". Reported data on the efficacy of AQ10 are contradictory (Sztejnberg, 1993). In some experiments, good control of powdery mildews of various crops was achieved, but other trials showed that the biocontrol partially or totally failed, although parasitism of pathogen colonies on the treated crop did occur. There are several biotic and abiotic factors that negatively affect the consistency of activity of *A. quisqualis* against PM. High temperature and low relative humidity represent two important limiting factors in biocontrol efficacy. Repeated applications are generally necessary. High humidity and rainfall promote the spread and develop of the mycoparasite.

The objective of the present work was to verify the presence of natural strains of *A. quisqualis* in a wide viticulture area (Northern Italy; Trentino Alto Adige region). Secondly, we aimed at isolating new strains that are better adapted to the local environmental conditions than commercial strain (AQ10) and highly aggressive against *Erysiphaceae*. The final aim is to develop a more effective biocontrol agent.

Material and methods

The effectiveness of the commercial strain (AQ10) in reducing PM infections was compared to other biocontrol agents (BCAs) in two greenhouse trials carried out with grapevine seedlings during 2008.

Two week-old plants were inoculated with powdery mildew by spraying a suspension of distilled water containing $1x10^6$ conidia/ml. The same plants were treated with AQ10 (Intrachem Bio, Italy), some microorganisms (filamentous yeasts and bacteria) and with natural substances (resistance inducer, potassium salt, plant and milk extract), which have already been reported to be effective against PM (Table 1). Plants were treated with sulphur (Thiovit, Syngenta Crop Protection, Italy) and distilled water as standard and untreated control, respectively.

The percentage of infected area with whitish, powdery spots on the upper surface of the leaf (disease severity) was visually assessed seven days after treatments.

Table 1: Microbial agents and natural substances tested in two trials in greenhouse (2008)

Designation	Active ingredient	Dosage
Sulphur	Sulphur 80%	3 g/l
Water	Distillated water	-
AQ10	*A. quisqualis*	0.12 g/l
Y1,2,3,4	Filamentous yeasts	10^{11} cfu/l
B1,2,3,4,5,6,7,8	Bacteria	10^{11} cfu/l
S1	Salt of fatty acids	10 ml/l
S2	*Melaleuca alternifolia* (plant extract)	10 ml/l
S3	Lactoperoxidase	1.5 g/l
S4	*Reynoutria sachalinensis* (plant extract)	10 ml/l
S5	Acibenzolar S methyl	0.1 g/l

The occurrence of natural parasitism of grapevine PM chasmothecia by *A. quisqualis* was assessed in Trentino-Alto Adige region in order to identify new *A. quisqualis* strains. The sampling was carried out from 2004 to 2007 in 27 vineyards. The size of each sampled vineyard ranged between 800 and 1200 m^2. In each vineyard and year, four replicates of 25 leaves each were randomly collected always sampling the 5th leaf from the top of the shoot. On each leaf mycoparasitism of *E. necator* was assessed under a light microscope in terms of presence of parasitized dull, flaccid and fawn-colored chasmothecia. Chasmothecia were mounted in lactophenol and the percentage of parasitized cleistothecia was assessed. Some parasitized chasmothecia were gently crushed by pressing the cover slip over the glass slide on which the sample was spread, to allow the release of conidia and pycnidia. Conidia were transferred on potato dextrose agar on Petri dishes (isolation of strains). The incidence of *A. quisqualis* in each vineyard was calculated (percentage of *E. necator* chasmothecia parasitized by the mycoparasite).

Ampelomyces quisqualis strains on parasitized chasmothecia and/or mycelium were initially identified by comparing the morphological characteristics with those described in the literature (Falk *et al*, 1995; Kiss, 1998; Kiss *et al*, 2004). A wide collection of *A. quisqualis* strains was made.

To evaluate the ability of *A. quisqualis* strains to reduce the disease we applied the mycoparasite on PM spots at the beginning of their appearance on the host plant. We evaluated pathogenicity, virulence and host range on

Podosphaera xanthii, *E. necator* and *P. aphanis* on cucumber, grape and strawberry plants. Cucurbit PM (*P. xanthii*) on cucumber was used as fungal host to evaluate the aggressiveness of the strains of *A. quisqualis* under controlled conditions (18-20°C and 70% RH). Two week-old plants were inoculated with PM by spraying a suspension of distilled water containing $1x10^8$ conidia/l. Once PM spots were sporulating (seven days after infection) a suspension of distilled water containing $1x10^9$ conidia/l of *A. quisqualis* was sprayed on plants. Plants with *A. quisqualis*-treated PM spots and untreated control (plant with PM spots treated with water) were kept for 48 hrs at 18-20°C and 95% RH. Ten days after *A. quisqualis* inoculation the number of PM conidia per leaf surface was counted (Thoma's hemocytometer). Results were expressed as reduction (percentage) of powdery mildew conidia in comparison to the untreated control. Analysis of variance was applied on "arcsin" transformed data and significant differences among treatments were separated by Tukey's test (α=0.05).

Results and discussion

Good results were obtained with some new microbial agents. The yeast Y1 and the bacteria B7 strongly reduced PM severity on grapevine leaves (Figure1).

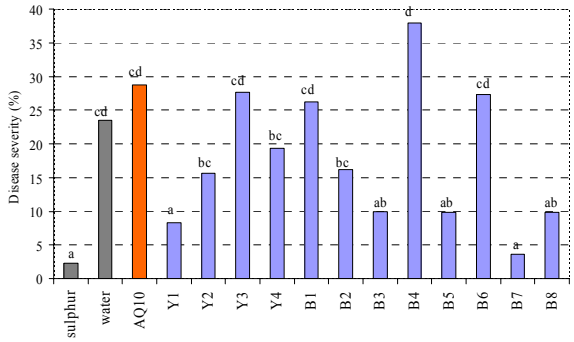

Figure 1: Comparison of the PM severity on grapevine leaves sprayed with *Ampelomyces quisqualis* (AQ10) and other microorganisms. Columns with the same letters are not significantly different (Tukey's test, α=0.05).

In the second experiment all the natural compounds as well as the two tested microorganisms significantly reduced PM infections on leaves (Figure 2). In both the experimental treatments with *A. quisqualis* (AQ10), had little effect and did not significantly reduce the disease.

The results of the vineyard-survey underline the generally low natural presence of *A. quisqualis* in the vineyards of the Trentino-Alto Adige region (Figure 3). The total number of chasmothecia parasitized by *A. quisqualis* significantly increased from 3 to 30 per sampled vineyard during the studied period (2004-07).

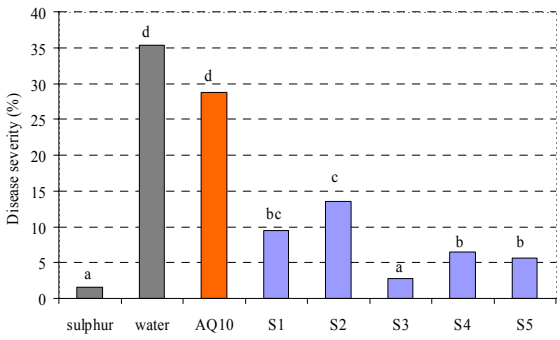

Figure 2: Comparison of the PM severity on grapevine leaves sprayed with *Ampelomyces quisqualis* (AQ10) and natural substances (S1-5). Columns with the same letters are not significantly different (Tukey's test, α=0.05).

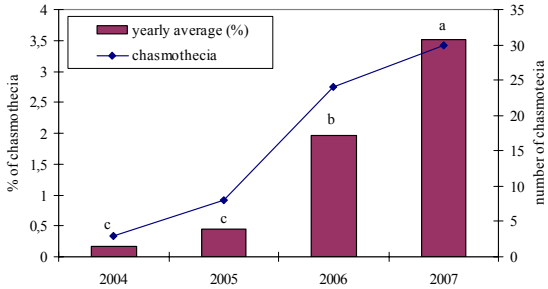

Figure 3: Average numbers per sampled vineyard (♦) and percentages of chasmothecia parasitized by *Ampelomyces quisqualis* (histogram) on grapevine in Trentino Alto Adige region in 2004-2007. Columns with the same letters are not significantly different ($P \leq 0.05$, Ryan's test).

The average rate of parasitism among chasmothecia in all of the monitored vineyards ranged from 0.17% (2004) to 3.51% (2007). When the mycoparasite was found in a certain vineyard, it was also detected in the following years, with increasing parasitization rates during the studied period.

In Trentino-Alto Adige we found approximately 200 *A. quisqualis* strains, but many of them showed similar cultural characteristics on potato dextrose agar and their conidia and pycnidia morphology were very similar. Therefore, we selected only three representative strains (ITA 1, ITA 2, ITA 3) for further analyses. These three strains and in addition 19 *A. quisqualis* strains isolated from different crops and PM species obtained from several culture collections (ATCC, CABI, DSMZ, CBS) were used for the comparison of efficacy. Each strain was named with a code (Table 2).

Table 2: *Ampelomyces quisqualis* strains collected from Trentino-Alto Adige region (Italy) and culture collections.

Designation	Site	Host plant
ATCC 245, 249, 250 ITA 1, 2, 3	USA Italy	Grapevine
CBS 128, 129, 130, 131 DSM 2222	Canada Germany	Cucumber
MYA 3389, 3391 MYA 3394, 3395 MYA 3398, B43	Hungary UK Germany	Apple
AQ10 DSM 2225 DSM 4624 CABI 272851 MYA 3401	Israel Germany Germany Ecuador UK	Wild and ornamentals species

The 22 strains of *A. quisqualis* were evaluated for pathogenicity, virulence, and host range in greenhouse assays using different plants and PM species. The obtained results showed that all tested isolates significantly reduced PM infection on grapevine (data not shown), strawberry (data not shown) and cucumber (Figure 4). The CBS group isolated from cucumber (Canada), the ITA group from grapevine (Italy), DSM 4624 isolated from *Leontodon* sp. (Germany) and CABI 272851 isolated from *Schinus molle* (Ecuador) highly reduced the number of PM conidia (by 88, 79, 77 and 79%, respectively). AQ10, DSM2222 (cucumber, Germany), the MYA group (apple, UK) and MYA 3401 (*Hydrangea macrophylla*, England) provided significantly lower reduction of *P. xanthii* conidia (22 to 63%). Very poor mildew reduction was obtained using the three ATCC strains from grapevine, New York (32-37% reduction).

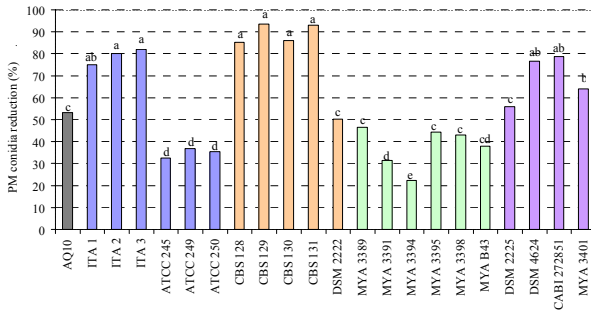

Figure 4: Reduction in the number of PM conidia of *Podosphaera xanthii* on cucumber leaves sprayed with *Ampelomyces quisqualis* strains coming from different host plant (different colour of the histogram) and geographic origin. Columns with the same letters do not significantly differ (Tukey's test, α=0.05).

Conclusions

The commercial strain (AQ10) is poorly effective against grapevine PM and several natural substances or microorganisms have better control activity. The use of a more aggressive strain of *A. quisqualis* may improve field performances of this biofungicide. Current studies underline that *A. quisqualis* is widely distributed in the vineyards of the Trentino Alto-Adige region. However, the level of natural parasitism on PM is low. Morphological characterization of different strains found in this region and of strains from culture collections revealed that there is no differentiation of *A. quisqualis* related to the geographical area of origin. Experiments with 22 *A. quisqualis* strains from different fungal hosts and geographic origin showed that they all reduce the sporulation of *P. xanthii,* but individual strains strongly differ in their aggressiveness against PM. Highly aggressive strains, more effective than AQ10, were identified which have a good potential for powdery mildew control since they reduced sporulation of the pathogen on cucumber by more than 90%. This result may represent the starting point for the improvement of efficacy of biofungicides based on *A. quisqualis*.

Acknowledgements

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Alternatives to copper for controlling grapevine downy mildew in organic viticulture

S. Dagostin[a], H. J. Schärer[b], L. Tamm[b], I. Pertot[a]

[a]IASMA Research and Innovation Centre, Fondazione Edmund Mach, via Mach 1, 38010 S. Michele all'Adige (TN), Italy, [b]Research Institute of Organic Agriculture FiBL, Ackerstrasse, Frick, 5070, Switzerland

Plasmopara viticola, an obligate biotroph belonging to Oomycetes, is one of the most devastating pathogens of grapevine worldwide. In organic agriculture grapevine downy mildew is still mainly controlled by regular sprays with copper compounds.

In Europe the use of copper fungicides in organic agriculture is restricted (Italy, France and Spain, Germany, Austria and Switzerland) or prohibited (Scandinavia and the Netherland). Over the last decade, research of more sustainable alternatives that comply with the principles of organic farming, has been a high priority of European policy.

This study is part of a European project (REPCO, 2004-2007) aiming at screening a wide range of substances to identify compounds that are effective against grapevine downy mildew and have a better eco-toxicological profile than copper.

Material and methods

Substances from animal origin (AN), biocontrol agents (BCA), inorganic materials (IM), microbial extracts (ME), natural derivatives (ND), plant extracts (PE), physical methods (PM), materials of synthetic origin (SY), and mixtures of substances from the previous groups were tested for their efficacy in grapevine downy mildew control. A total of 113 substances at several dosages were considered.

Preliminary evaluation of the efficacy in controlling grapevine downy mildew was carried out under semi-controlled conditions with artificial inoculation. Substances, which were as effective as copper, were then tested under natural field conditions in presence of natural infection.

Experiments under semi-controlled conditions were performed in greenhouses and growth chambers in S. Michele all'Adige (Italy) and Frick (Switzerland). Potted plants (4-10 leaves) of susceptible grapevine (*Vitis vinifera*) cv. Pinot Gris and cv. Chasselas were used. The plants were sprayed with the test preparations, left to dry at room temperature and inoculated. The inoculum (water suspension of 5×10^5 *P. viticola* sporangia ml^{-1}) was sprayed on the abaxial surface of each fully expanded leaf.

Inoculated plants were incubated in humid chamber for 12 hour at 20°C, 99% relative humidity (RH), in the dark and then placed under greenhouse/growth chamber conditions. Six to ten days after inoculation, plants were incubated to promote sporulation on oils spots (20°C, 99% RH, dark) and thus enhance assessment.

The field trials were conducted from 2004 to 2007 in Italy on cv. Cabernet Sauvignon, and in Switzerland on cvs. Riesling-Sylvaner and Chasselas. The experimental design consisted of four replicates, with an area of approximately 18 m^2 and six to eight plants each, arranged in a completely randomized block design. The treatments were applied at weekly intervals.

Each experimental set (indoor and field trial) included an untreated control (water) and a standard treatment (copper hydroxide, Kocide 2000, 1.42 g/l; DuPont de Nemours, USA).

Both in greenhouse and field trials disease presence was assessed as disease incidence (percentage of leaves or bunches with downy mildew symptoms) and disease severity (percentage of leaf or bunch area with downy mildew symptoms). Symptoms and possible phytotoxic effects of the experimental treatments were assessed based on EPPO standard scale (EPPO, 2004). The effects of the different treatments were analyzed using ANOVA and multiple mean comparison tests. In all experiments, the treatments were compared to the untreated control and the standard treatment. Results were divided in classes: (+) effectiveness not different from copper and different from water reference in both greenhouse trials (IT and CH) or in all years; (+/-) effectiveness different from copper and different from water reference or not consistent among locations/years, (-) effectiveness different from copper and not different from water reference in both greenhouse trials (IT and CH) or in all years.

Results

In indoor trials, 219 combinations of products and dosages were evaluated. Disease severity, as compared to the untreated control, was significantly reduced by 63.4% of the substance/dosage combination treatments, and 36.5% of the substance/dosage combinations were as effective as the copper standard. Based on these results the activity of 24% of the substances was further tested under field conditions (Table 1).

In spite of the high efficacy shown in indoor trials, the effectiveness of natural products under field conditions was often low. An explanation of the lower efficacy compared to indoor trials can be found in their fast inactivation/decomposition by environmental factors (low humidity, pH and UV radiation), rainfastness, and uneven leaf coverage.

On leaves, under field conditions, inorganic material (new formulation with low dosage of copper), fatty acid and synthetic compounds provided a high level of protection against *P. viticola*. Among the other products good results were obtained with saponin derivates from *Yucca schidigera*, but not from Q*uillaja saponaria* and *Camellia oleifera,* and sage extracts. Physical method did not control the disease.

Table 1: Efficacy trials conducted in 2004-2007 under greenhouse and field conditions in Italy (IT) and in Switzerland (CH). Listed products were selected from 113 products tested in several trials. Product activity is displayed by class: (+) effectiveness not different from copper and different from water reference; (+/-) effectiveness different from copper and different from water reference, or not different from copper and not different from water reference, (-) effectiveness different from copper and not different from water reference. Significant difference ANOVA and Tukey's test at p-level < 0.05. **L**: leaves, **B**: bunches

				Greenhouse	Field			
	Active ingredients	**Commercial**	**Dosage**	**IT/CH**	**Italy**		**Switzerland**	
					L	**B**	**L**	**B**
AN[1]	Chitosan	Chitoplant	1.0 g/l	+/-	+/-	+/-	+/-	+
	Lactoperoxidase	Enzicur	1.5 g/l	+/-	+/-	+		
BCA	*Trichoderma harzianum* T39 + *Clonostachis rosea*	Clonotri	0.6 g/l	+	+/-	+/-		
	Bacillus subtilis	Serenade	4.0 g/l	+/-	+/-	+/-		
	Bacillus pumilis	Sonata	4.0 g/l	+/-			-	-
	Trichoderma harzianum T39	Trichodex	4.0 g/l	+	+/-	+/-		
IM	Potassium bicarbonate	Armicarb	5.0 g/l	+			+/-	+
	Copper gluconate (8% Cu^{2+})	Labicuper	3.0 ml/l	+	+	+		
	Sulphurated clay, *Equisetum arvense*	Myco-sin	5.0[2] g/l	+	+/-	+/-	+	+
	Copper peptidate (25.6% Cu^{2+})	Naturam 5	4.0 ml/l	+	+	+		
ME	*Pseudomonas aureofaciens*	Agat 25-K	10 ml/l	+/-	+	+	-	-
ND	Fatty acid	Tecnobiol	10 ml/l	+	+/-	+	+	+/-
PE	*Melaleuca alternifolia*	BM-608	5.0 ml/l	+	-	+/-	-	-
	Plant-based alcohol extract	Elot-vis	5.0 ml/l	+/-	+/-	+		
	Solidago virgaurea	Experimental	50 ml/l	+	+/-	+		
	Salvia officinalis	Experimental	50 ml/l	+	+/-	+		
	Inula viscosa	Inulex	5.0 ml/l	+	+	+	+/-	+/-
	Abies sibirica	Novosil	5.0[3] ml/l	+	+/-	+	-	-
	Quillaja saponaria	Quiponin SB	50[4] ml/l	+	-	+	-	+/-
	Salix alba	Salix extract	20 ml/l		+/-	+/-		
	Yucca schidigera	Saponin	10 ml/l	+	+/-	+	+/-	+/-
	Camellia oleifera	Teawet TQ	50 ml/l		-	+	-	+/-
	Melaleuca alternifolia	Timorex	10 ml/l	+	+/-	+	+	+/-
PM	Ozonated water + UV irradiation	PhytO3	-	-			-	+/-
SY	Fosetyl-Al	Aliette	2.5 g/l				+	+
	Beta-aminobutyric acid	BABA	1.0 g/l	+	+/-	+/-	+/-	+/-
	Acibenzolar-S-Methyl	Bion 50 WG	1.0 g/l	+	+	+		
	Phosphorus pentoxide, potassium oxide	FosfiD'or	3.0 g/l		+	+		

[1]AN animal origin, BCA biocontrol agent, IM inorganic material, ME microbial extract, ND natural derivative, PE plant extract, PM physical method, SY synthetic origin
[2]Serenade was used at concentration 8 g/l in field trials carried out in Switzerland
[3]Novosil was used at concentration 10 ml/l in greenhouse trials
[4]Quiponin SB was used at concentration 25 ml/l in field trials carried out in Switzerland

Tea tree extract efficacy was highly influenced by formulation; in fact, the experimental product BM-608 did not control disease infection, while the commercial product Timorex allowed good disease control. All the other tested compounds reduced disease symptoms on leaves less than copper, but they were different from water control. However, plants treated with chitosan or *Inula viscosa* at high rates, showed phytotoxicity on the leaves in the field trials in Switzerland and Italy, respectively.

Assessments on bunches indicated that, with the exception of *Abies sibirica* extract, *Melaleuca alternifolia* (tea tree), and *Bacillus pumilis*, all products were able to control downy mildew infection as well as copper hydroxide.
Numerous alternatives to copper for use against downy mildew under both controlled conditions and under field conditions, at two locations over four years were evaluated. Even though commercial products that can fully replace copper in organic viticulture are not presently available, the results of this study may help researchers and farmers integrate less effective products into copper-based disease control programs in order to reduce the quantities of copper applied in organic vineyards.

Acknowledgments
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Session 4: Posters

What is Life cycle management for grapevine fungicides?

M-P. Latorse

Research Biology Fungicide, Bayer Cropscience, BP9163, 69263 Lyon Cedex09, France,
marie-pascale.latorse@bayercropscience.com

The objective of life cycle management for fungicides is to offer customers a long term strategy for plant diseases control such as grapevine downy and powdery mildew, including sustainable technical solutions through adapted use recommendations. Laboratory and field integrated studies are conducted to define and adapt anti-resistance strategies, optimise product positioning as well as to design and characterize new formulations.

From the early phase in the development process, the risk of resistance occurrence and evolution is investigated and a management strategy should be implemented before resistance becomes a problem. A precondition for effective resistance management is the establishment of baseline data before introduction of the fungicide on the market. This consists of random monitoring over the main vineyards followed by a sensitivity characterisation in laboratory tests. In parallel, long term trials are initiated in order to demonstrate, over several years of selection pressure at the same location, the efficiency of anti resistance strategies. After market introduction, sensitivity of the pathogen populations is annually monitored, in practical conditions to detect any potential shift in sensitivity. Once resistant or less sensitive strains have been identified, laboratory and field trials are carried out to check fitness of these resistant isolates. Cross resistance profiles are also established with other active ingredients belonging to chemical classes showing a different biochemical mode of action. Absence of cross resistance between two active ingredients means that the corresponding mixture is suitable to manage the risk of resistance development.

On the other hand, the biological mode of action as well as the intrinsic features (such as preventive or curative action, long lasting activity, rainfastness, chemo dynamics) depending on physio-chemical properties of the active ingredients and their formulations are examined to define strengths and weaknesses for a proper positioning of the product within treatment programmes. The objective of these studies is to propose more effective and easy to use solutions for the market. During the life cycle of the product, complementary experiments are regularly carried out to optimise this positioning according to introduction of new fungicides into the market, more stringent regulations, new consumer demands and new findings in pathogen population structure. Additionally, beneficial effects of fungicide compounds are investigated especially in regards to the quality of production (reduction of mycotoxins, effect on late season diseases)

In conclusion, life cycle management of a grapevine fungicide is a continuous process designed to provide better solutions to answer the problems of the customers.

Efficacy of sprays applied during the 'open window' period of susceptibility of grapevine powdery mildew (*Erysiphe necator*)

WK. Kast, K. Bleyer

Staatliche Lehr- und Versuchsanstalt fuer Wein- und Obstbau, Traubenplatz 5, 74189 Weinsberg, Germany

Summary

From 1999, sprays were carried out as field trials that included a comparison of two spray regimes differing in the amount and point in time of fungicide application. The comparison included Regime 1, with three sprays starting just before flowering, during blossoming and at berries size 2mm and Regime 2, with seven sprays which include the three sprays of Regime 1 plus one earlier and three later sprays. The same results were achieved in nine of 10 years. Even under extreme disease pressure, three sprays had more than 90% of the preventive effect of seven sprays. In 2002, we could not spray the trial during the period one week before flowering until the end of blossoming due to an accident with the experimental plot sprayer. Treatments in Regime 1, with three sprays, showed only 40% efficacy in this case but Regime 2, with seven sprays, did not increase the level of efficacy achieved.

Introduction

Investigations by Stark-Urnau and Kast (1999) indicated that the developing fruit of grapevine are exclusively highly susceptible one week before blossoming until berries are 2mm. Even highly susceptible varieties like Trollinger (syn. Venatsch) are mainly infected with severe infections in this period. Since 1999, fungicide sprays were applied in field trials that included a comparison of two spray regimes differing in the amount and timing of sprays.

Material and methods

Three wine varieties *viz.* Trollinger, Silvaner and Cabernet Dorsa (Table 1), of differing susceptibility to powdery mildew, were used for the trials in the different years. Field trials were carried out with plots of 12 vines in one row separated by untreated rows on both sides, with four replications in a randomised block design. The fungicides were applied with a experimental tunnel-sprayer that allowed precise treatment of single rows. In most years natural initial inoculum was present, because untreated rows of the previous year were used for the experiments. In the years 2008 – 2009, the initial inoculum was increased by the addition of powdery mildew on infected vines grown in the glasshouse in 12 cm diameter pots. These vines carried about 10 leaves completely infected with powdery mildew. During the stage of three leaves unfolded (BBCH13), two infected pots per plot were fixed on a trellis of the experimental plots.

The trials included two spray regimes. Regime 1 with three sprays starting about one week before flowering (BBCH55), during blossoming (BBCH64-66) and at berries size 2mm (BBCH73); and Regime 2, with seven sprays including the 3 sprays of Regime 1 plus one earlier spray using wettable sulphur, and three later sprays using different triazol-fungicides and other powdery mildew fungicides.

In 2002, an accident of the experimental-plot-sprayer during the first spays caused a lot of damage. Until the repair period of this machine we could not spray all trials. This caused a gap in the spraying schedule during the period one week before flowering till the end of blossoming (BBCH55-69).

Figure 1: An accident with spray application equipment in 2002 during OWP caused special, but

Table 1: Fungicides used for the trials in the different years (for OWP*)

Year	used at BBCH55	used at BBCH65	used at BBCH73	vine variety
1999	Discus	Discus	Discus	Silvaner
2000	Vento	Vento	Vento	Silvaner
2001	Flint	Flint	Flint	Silvaner
2002§	Flint	Flint	Flint	Silvaner
2003	Flint	Vento	Flint	Trollinger
2004	Flint	Discus	Vento	Trollinger
2005	Topas	Vento	Collis	Trollinger
2006	Flint	Flint	Flint	Cabernet Dorsa
2007	Talendo	Cabrio Top	Talendo	Cabernet Dorsa
2008	Talendo	Cabrio Top	Talendo	Cabernet Dorsa
2009	Talendo	Flint	Cabrio Top	Cabernet Dorsa

* OWP: Open window period (period from BBCH55-73)

§ a gap was caused by an accident with the spraying equipment

All fungicides (table 1, wettable sulfur and all trazol-fungizides) were applied at the recommended dose for the relevant phenological stage. Powdery mildew

disease severity was scored on 100 grapes per plot at veraison and 400 leaves per plot in the last week of August.

Results and discussion

Even under extreme disease pressure, three fungicide applications provided nearly the same preventive effect as did seven sprays (Table 2). The maximal difference in the reduction of the disease (RW%) was found in 1999 (12% difference). In 2002, technical problems with the tunnel sprayer caused a gap in the spray schedule of 18 days beginning 10 days before flowering (BBCH54). The next spray in all treatments was at the end of blooming period (BBCH69). The effect of three sprays (RW%) was only 40% in spite of a relatively low disease pressure in this year. Yet, seven sprays had the same low effect. This shows that gaps in this period are extremely risky and infections could not could not be cleared by the further sprays.

The period starting one week before flowering and ending a few days after berries are 2 mm diameter (period from BBCH55-73) was named as "Open-Window-Period" (OWP). During OWP winegrowers need an absolutely sure powdery mildew spray program. They therefore should use the best fungicides in this period. Also, during OWP, winegrowers need to work very accurately against powdery mildew, especially if the weather conditions are suitable for powdery mildew.
If the OWP fungicides against powdery mildew should be used in short spray intervals, especially if the weather conditions are very suitable for the fungus, all sprays at other times have a very low relevance for the final result. These facts were integrated into the expert system OiDiag as an ontogenetic part index.

Table 2: **Effect on disease severity of applying fungicides in the window of susceptibility for grape powdery mildew, in relation to untreated controls (RW%) 1999-2008.**

	Untreated	Regime 1 Infection Window (3 sprays)		Regime 2 Complete Schedule (7 sprays)	
Year	**Severity (%)**	**Severity (%)**	**RW (%)**	**Severity (%)**	**RW (%)**
1999	42	9	78,6	4	90,5
2000	20	2	90,0	1	95,0
2001	18	0	100,0	0	100,0
2002	13	7§	46,2	7§	46,2
2003	0	*	*	*	*
2004	9	0	100,0	1	88,9
2005	1	0	100,0	0	100,0
2006	0	*	*	*	*
2007	22	1	94,5	0	98,6
2008	62	2	96,8	1	98,4
2009	65	2	96,9	1	98,5
Mean	23	2,6	88,7	1,9	92,6

*: not assessed, disease incidence in untreated not relevant
§ a gap in spray schedule caused by an accident with the experimental plot sprayer from BBCH55 to BBCH69

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Antifungal activity on grapevine downy mildew (*Plasmopra vitcola*) of the ethanol extract of *Salvia officinalis* and its components

S. Dagostin, O. Giovannini, S. Carlin, I. Pertot

IASMA Research and Innovation Centre, Fondazione Edmund Mach, via Mach 1, 38010 S. Michele all' Adige TN, Italy

Introduction

Aromatic plants and herbs are known as potential sources of natural compounds with medicinal and antimicrobial activity. Pharmacological properties of plants belonging to *Salvia* spp. have been investigated and their use in food, flavoring, perfumes and drugs are well documented. Moreover, preliminary *in planta* studies demonstrated that essential oils and organic extracts of *S. officinalis* have some disease control properties against phytopathogens like *Pseudoperonospora cubensis* (cucumber downy mildew), *Peronospora destructor* (onion downy mildew), *Bremia lactucae* (lettuce downy mildew), and *Phytophthora infestans* (tomato late blight) (Dorn *et. al* 2007, Nowak *et al.* 2009, Schmitt *et al.* 2008).

Plasmopara viticola (Berk. & Curt.) Berl. & de Toni, which is the causal agent of grapevine downy mildew, attacks leaves and bunches, negatively affecting both quantity and quality of crop yield. At present, copper is the most important fungicide in organic agriculture, but it has recently come under eco-toxicological scrutiny because of its accumulation in soil. Therefore reducing copper applications and/or identifying alternative treatments are the major priority for the future.

Our objectives were to evaluate the efficacy of an *S. officinalis* L. extract against grapevine downy mildew under greenhouse and field conditions, the persistence of its disease control, and the rainfastness on plants. Moreover the chemical nature of extract components and their putative fungicide activity were evaluated.

Material and methods

The alcoholic extract (100%) was prepared by Soxhlet extraction with 99.8% ethanol of Dalmatian sage (*S. officinalis)* dried leaves.

The residue was subjected to column chromatography (CC) on silica gel, eluting the column with a chloroform methanol gradient. Fractions were collected and concentrated to dryness under reduced pressure. A total of 32 fractions were collected.

Preliminary gas chromatography (GC) analyses of fractions were performed with Trace chromatograph equipped with a capillary column SOLGEL-WAX™ (30 m length × 0.25 mm ID × 0.25 µm film thickness). The GC oven temperature was set at 50°C for 3 min, increased to 220°C at rate of 4C°/min and to 260°C at rate 10C°/min, and the final temperature was kept for 20 min. The temperature of the injector and detector were set to 250°C. The flow rate of carrier gas (He) was 1.2 ml/min.

The percentage composition of the extract was computed from GC peaks areas. The qualitative analysis was based on the comparison of retention index with corresponding data in NIST 98 and Wiley libraries.

Greenhouse evaluations of sage extract activity, persistence of activity and rainfastness were carried out on *Vitis vinifera* (cv. Pinot gris) potted plant. The evaluation of control activity of fractions obtained from sage extract CC, was carried out on *V. vinifera* (cv. Pinot gris) rooted cuttings. In the evaluation of control activity the treatments were applied 6 h before inoculation. To evaluate the persistence of activity over time, the treatments were applied once at 6, 3, 2, or 1 day or 6 h before pathogen inoculation. To evaluate the rainfastness of the extract the treated plant were subjected to 0, 10, 30 or 50 mm of simulated rain before pathogen inoculation.

Grape plants were inoculated by spraying the abaxial surface leaf with the *P. viticola* sporangia suspension (10^5-10^6 sporangia/ml).

The activity of the molecules identified by GC was tested on leaf disks cut from one-two week old grapevine leaves (cv. Pinot gris). Solutions of single or combined molecules were sprayed on disks with a spray gun, and *P. viticola* sporangia suspension (inoculum) was applied with Potter laboratory spray tower. Leaf disks were incubated at 20°C and 100% relative humidity for five days and then the presence of sporulation was assessed.

To evaluate the efficacy of the sage extract for controlling grapevine downy mildew under natural environmental conditions, field trials were carried out in Northern Italy (Trentino region) in 2006 and 2007 on cv. Cabernet Sauvignon. Treatments with the extract and assessment of disease were carried out weekly.

Sage crude extract and CC fractions were applied in greenhouse and field trials at a final concentration of 50 ml/l. Copper hydroxide (Kocide 2000; 1.42 g/liter; Du Pont de Nemours, USA) and water were used as the standard treatment and control, respectively.

The activity against downy mildew on leaves (greenhouse and field experiments) was assessed as disease severity, (percentage of leaf area showing signs of infection) whereas the activity on bunches (field experiments) was assessed as disease incidence (percentage of bunches showing signs of infection). Data were subjected to ANOVA and Tukey's test ($\alpha = 0.05$).

Results

In each experiment conducted under greenhouse conditions, sage extracts exhibited a high level of preventive activity with an efficacy ranging from 79.9-95.8%, similar to that of copper hydroxide (80.5-96.0%).

The significant effect of the sage extract and copper on grapevine downy mildew did not decreased for up to 6 days after product application. In the rainfastness study, sage extract showed a high level of disease control (about 80%) without rain, but the level of disease control decreased with rain application (Figure1).

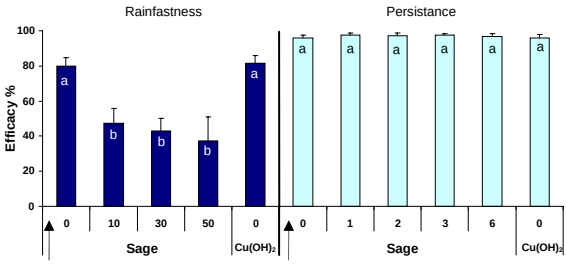

Figure 1: Efficacy of *Salvia officinalis* extract and $Cu(OH)_2$ against severity of downy mildew on leaves of potted grapevine. Reduction in disease was related to the untreated control according the equation 100 (y_U-y_T) y_{U-1} with y_U disease severity in the water control and y_T levels of severity in the treated plots. Treatments with a letter in common are not significantly different (ANOVA and Tukey's test, $\alpha = 0.05$). Error bar = standard error.

Under field conditions sage extract caused a reduction in AUDPC for disease severity on leaves both in 2006 and 2007 and in incidence of disease on bunches in 2006, not significantly different from those obtained by copper hydroxide.

Efficacy evaluation of fractions obtained from separation of sage extract on silica gel column led to the selection of four, out of 32, fractions with activity against *P. viticola.*

In the first experiment the combination of fractions 2+3, and 4+5, showed a high control of *P. viticola* significantly different from water and similar to copper (Figure 2). Fractions 10 and 7 show a quite reduction of disease even if not different from untreated control. Further experiments confirmed that second to fifth fraction (applied solo and in combination), eluted with chloroform a rich mobile phase ($CHCl_3$:MeOH 20:1 and 15:1), and allowed high reduction of grapevine downy mildew on potted plants.

The active fractions (2-5) were analyzed by GC (Figure 3) and their constituents were identified.

Based on the comparison of retention index with corresponding library data, the seven major components of active fraction were identified as thujone, camphor, caryophyllene-like compound, borneol, globulol, and two similar compounds codified as COD-1 and COD-2. Other minor constituents were 1, 8-cineole (eucalyptol) and α-humulene.

In the first leaf disk experiment the assessment of sporulation highlighted that only globulol, COD-1, COD-2, and camphor at concentration of 100 ppm resulted in a mild decrease in level of pathogen sporulation. In the second experiment COD-1 and COD-2, tested at concentration of 1,000 ppm, totally controlled *P. viticola* while neither camphor nor viridiflorol, at 1000 ppm, inhibited pathogen sporulation.

Conclusion

The results obtained under controlled greenhouse conditions demonstrate that sage ethanol extract is a promising natural product for *P. viticola* control. The sage extract showed good persistence of action on grapevine leaves but its performance was strongly affected by rainfall and thus efficacy in organic vineyard, as shown in field trials, could be compromised. A formulation of sage extract with a high level of rainfastness is necessary for improving extract activity under any meteorological conditions.

Analysis of ethanol extracts of *S. officinalis* components indicated that the extract activity against grapevine downy mildew could be attributed to two molecules, COD-1 and COD-2.

Detailed chemical characterization and toxicological and eco-toxicological profiles of the active molecules are under investigation.

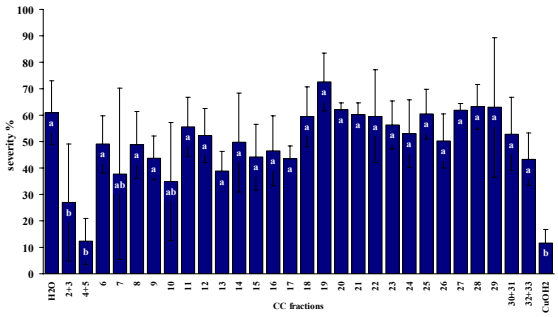

Figure 2: Downy mildew severity on grape rooted cuttings treated with fractions obtained from *Salvia officinalis* extract separation on silica gel CC. Fraction 1, stationary phase solvent, was not tested. $Cu(OH)_2$ was used as control. Treatments with a letter in common are not significantly different (ANOVA and Tukey's test, $\alpha = 0.05$). Error bar = standard error.

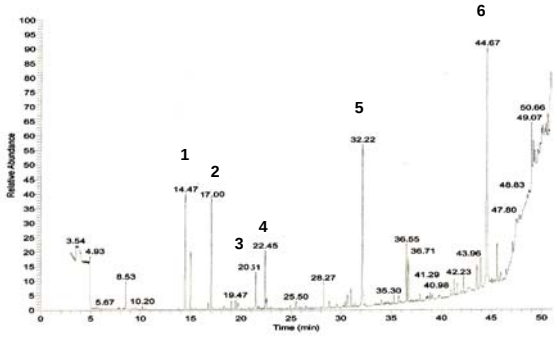

Figure 3: GC spectrum of major components of fraction 4 obtained from CC of *Salvia officinalis* extract. 1: thujone, 2: camphor, 3: caryophyllene-like compound, 4: borneol, 5: globulol, 6: COD-1 and COD-2 compounds.

Acknowledgements

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Intrinsic sensitivity of vine fields to downy mildew: Elaboration and validation of a decision support cartography.

D. Lafond[a] , E. Goulet[ab], D. Rioux[b], J. Marsault[a], M. Raynal[c]

[a] IFV Val de Loire – Centre, 42 Rue Georges Morel, 49071 Beaucouze, France, [b] Cellule « Terroirs Viticoles » (CTV), 42 Rue Georges Morel, 49071 Beaucouze, France, [c] IFV Bordeaux – Aquitaine, 39, rue Michel Montaigne, 33290 Blanquefort, France

Introduction

With the growing social pressure for environmental care in agricultural production, wine growers have to consider reducing their pesticides use. Several tools exist to give them information about the risk level to which they are exposed. Concerning fungal diseases, which are the main reasons for treatment in viticulture, these tools are mainly based upon climatic consideration, as climate variables (temperature, rainfall) are very pertinent to determine the disease incidence of the vintage. The modeling tools developed at the IFV are based on climatic variables, and great progress has been done on refining the scale of maps produced by these tools (Raynal *et al.*, 2008). However, secondary factors, related to the soil, the surrounding landscape or the planting material, are also known to intervene in the sensitivity of the vine. Those factors are characteristic of a small area of land. Knowledge about the impact of those secondary factors can be of a great help to wine growers if they could be precisely related to the disease sensitivity of a vine field.

Goulet *et al.* (2006) used an indirect methodology in order to evaluate the influence of each factor on vine sensitivity to downy mildew and powdery mildew, and to rank them. This method was based upon surveys, bibliography and expert interview. The survey of 3862 cultural units, crossed with the soil units database of the CTV (established through an exhaustive field study including soil characterization, landscape description and land-survey), led to the determination of the following hierarchy concerning downy Mildew :

Soil > Landscape > Planting material

Among the soil factors, the nature of the soil as described by Bodin et Morlat (2006) (Strongly Weathered Rock (SWR), Moderately Weathered Rock (MWR), Weakly Weathered Rock (WWR)), the water holding capacity (WHC) and the drainage (d) are the most relevant. A typology of soils in three classes using these criteria has been established (Figure **2**):

1 : Soil type = SWR *AND* { WHC ≥ 150 mm *OR* : d ≥ 4

3: Soil type = WWR *AND* { WHC ≤ 100 mm *OR* : d ≤ 3

2 : Others

Figure 2: Soil Typology : Soil Sensitivity Classes : 1 : High, 2 : Medium, 3 : Low ; Drainage : 1 :Very good, 2 : Good, 3 : Correct, 4 : Passable, 5 : Weak, 6 : Bad

Using this typology with the CTV's database allowed us to establish a cartography of intrinsic sensitivity to downy mildew for the regions of Anjou and Saumur (Figure 3).

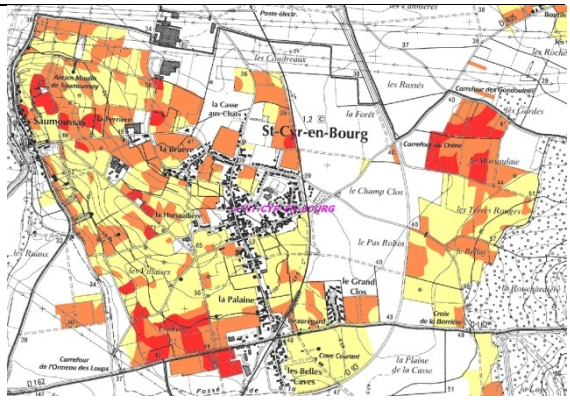

Figure 3: Map of sensitivity on the St-Cyr-en-Bourg area *(Loire Valley, France). Red : Class 1, Orange : Class 2,* Yellow : Class 3

This kind of map could be very useful for wine growers, but it must respond to the following criteria: First, it needs to be precise. Second point, the classes have to be different enough to justify a difference in the plant protection policy. And third, the classes have to be robust enough to be sure that a class 3 area really has a low sensitivity.

The method of soil characterization used by the CTV (Morlat *et al.*, 1999) ensures that the precision of the cartography is sufficient at the vine field scale. The aim of this study is to validate this cartography on the second and the third point, by mean of field experiment.

Material and methods

Selected cultural units were those where both class 1 and class 3 areas were present ; cartography and aerial views were used in a first step (Figure 4), then an on-field validation of the choice was performed. 9 plots were found in several topographic configurations and bed-rock types (limestone, schist), in the Anjou and Saumur regions.

For each soil class, two sets of 5 to 7 stocks in a row were selected. Plastic sheets were used to protect them during treatments. Downy mildew was evaluated weekly (frequency and intensity on leaves and bunches) between May 1st and August 15th, on 50 leaves and every bunch for each set of stocks. During winter, the weight of pruning woods was measured on each plot, on 50 stocks for the two different soil classes, in order to evaluate the vine vigor.

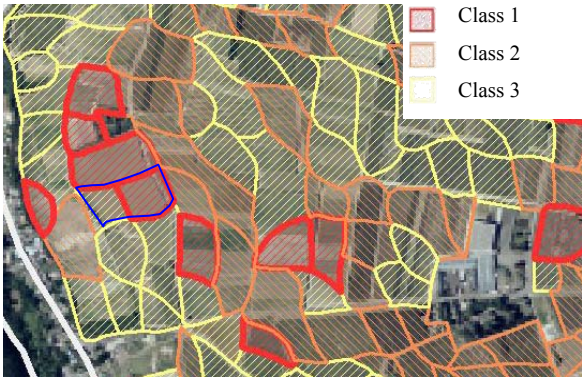

Figure 4: Situation of one experimental plot (in blue) on Chacé district (Loire Valley, France)

2009 results

The early symptoms appeared first on the high sensitivity part of the plot on two of the nine plots; they appeared first on the low sensitivity part on two others, and they appeared the same week on five plots.

The mean comparison (using t-tests) of the observation for each date gave the same type of contrasted results. In many cases, no differences could be seen. In one case, the downy mildew attack was significantly higher on the high sensitivity part than on the low sensibility part. On two other plots, it was the opposite (Figure 5).

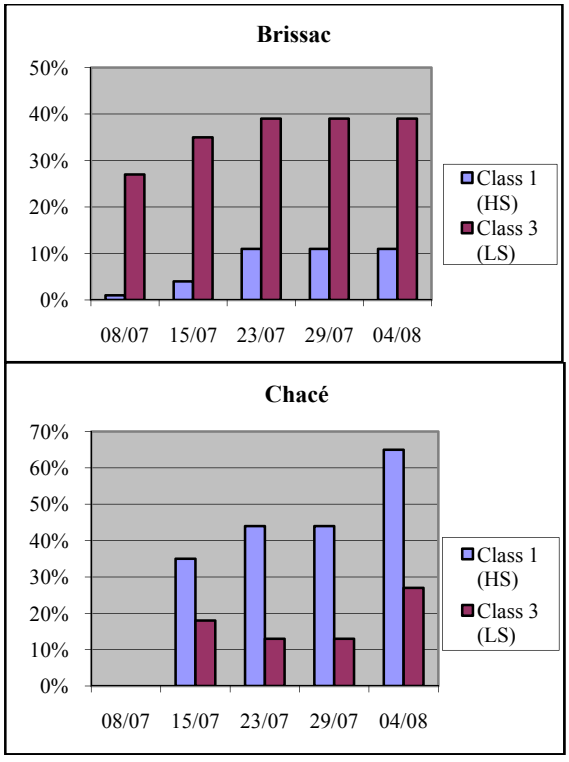

Figure 5: Evolution of downy mildew frequency on leaves on two plots (HS: High Sensitivity, LS: Low Sensitivity).

Discussion

There is no global trend that emerges of these results. However, there are different ways to explain them.

✓ The predominance of soil factors has been shown by Goulet *et al.* (2006) statistically, on 3862 cultural units. However, other factors may intervene in particular situations, and make this predominance only relative. Other factors (topography, local climatic conditions, etc…) may locally have a significant influence on downy mildew development. From a practical point of view, it means that the cartography, in its present state, would be useless for wine growers.

✓ Some methodological problems may explain those results. The design which was used didn't take into account the randomness of primary contaminations. With only two places of 5 to 7 stocks on each soil class, the chance of having a contamination at the first contaminating rain precisely in those places is weak, and contaminations may occur only in one of the places and not in the others, even on low sensitivity places. Those primary contaminations give an "advantage" to the places where they happened and this difference lasts until the end of the growing season.

Conclusion and outlook

2009 results can't allow us to conclude about the cartography validity. This trial is to be conducted again in 2010, with modifications in the design to avoid this artifact of "random contamination". Starting with a wide untreated area (more than 100 stocks) will allow us to set the follow-up places on the first contaminations, allowing us to follow the epidemic progression from the beginning. The results of this second year trial should allow us to conclude whether the cartography can be a useful tool for wine growers or not.

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Impact of radar pluviometry data on the modelisation of downy mildew in Bordeaux vineyards

M. Raynal[a], C. Debord[a], S. Guittard[a], M. Vergnes[a], K. Griaud[b], N. Fernandez[c], S. Strizyk[d], D. Boisgontier[e], J. Congnard[f], D. Grimal[f]

[a] IFV, Pôle Aquitaine 39, rue Michel Montaigne, 33290 Blanquefort, [b] : Faculté œnologie,Université Bordeaux II, 351 cours de la libération, 33405 Talence, [c] : ENSAT, Avenue de l'Agrobiopôle - BP 32607 Auzeville-Tolosane 31326 Castanet-Tolosan Cedex, [d] : SESMA : 40 rue des frères Flavien – 75020 Paris, [e] : CAP2020 : 2 allée du chemin neuf – 91720 Gironville sur Essone , [f] : Météo France - 7 avenue Roland Garros – 33700 Mérignac

The French Vine and wine Institute (IFV), is promoting lower chemical inputs through modeling of downy and powdery mildew in vineyards. The EPIcure Information System is a Decision Support System between Climate (weather station network), Parasite (mildew models) and Plant (untreated plot network). The GIS (www.vignevin-epicure.com) initial goal was to have risk evaluation at a small-scale region.

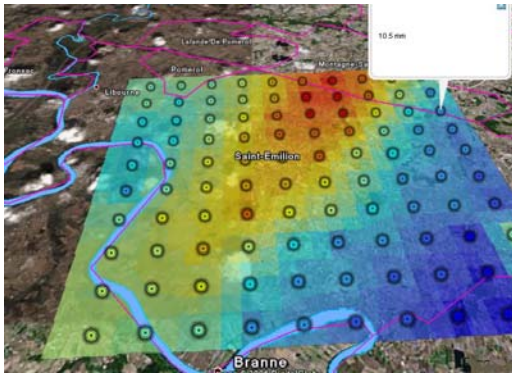

Figure 1: Model variation in short distance

The models use input rainfall and temperature measured by weather stations. Almost 50 weather stations are available in Bordeaux vineyards. Each week during the growing season, IFV advises vine growers if treatments are needed or not. IFV saves three or more treatments each year. Our objective was to further improve the models used by IFV.

"From point to surface" analysis

The spatial representativeness of the meteorological stations is limited: 50 stations in Bordeaux vineyards (120000 ha). To improve precision, interpolation was used to predict values from a limited number of sample data points. We increased the number of points with radar technology based on the reflectivity formula : $Z = a * R^b$

First Benchmark in 2006

The first test involved radar information from a rainfall radar (Hydrix) designed by French Novimet company. A rain dataset was used to simulate a mildew epidemic in Saint Emilion area. IFV used a square composed of 100 virtual stations. The model showed some differences between two separated points of a distance of four kilometres. This benchmark showed the model could be sensitive over short distances.

Automatization in 2007

IFV purchased data from Météo France. Antilope is a product that provides precipitation data for a surface about 1 km^2 (data based on radar for location and weather stations for calibration of the rainfall). Temperature was calculated by Safran product.

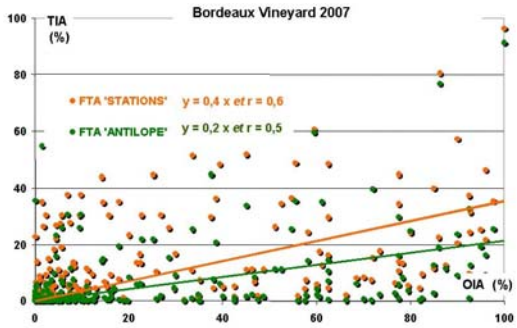

Figure 2: Theoretical incidence of downy mildew attack simulated with Antilope and weather station rainfalls compared to observed frequency of attack on untreated plot network.

Having one virtual pluviometer at a kilometer scale means IFV has 10 000 pluviometers at Gironde department scale resumed to 6000 precipitation data in Bordeaux vineyards Consequently, it is obvious we can see local phenomenon with radar technology. For instance, on May 24th 2007, a small area near village of Fougueyrolles was hit by a major rain event. The radar recorded a daily rainfall of 106.4 mm but the network of station only measured 8mm. IFV also observed a large difference in rainfall between two neighbour parcels (TIA on August 1st, 2007) exactly as shown by the model.

This one- year study showed good rainfall cell detection but the quantification of rain could be improved. There is still room between the simulated and observed data. Nevertheless, it gave an important impact on the project:

- good spatial representation of information
- historical weather data for future reference

New method for new data

IFV has been able to make a real progress in its disease forecasting. Unfortunately, this data is not precise enough to quantify rainfall.

• Improve radar calibration using more weather stations
• Test a new Radar technology (Radar Hydrix from Novimet society)

• Develop a more precise spatialisation of temperature (1km²)
• Improve monitoring inquire on attacked cells

IFV tested a protocol of investigation to ensure a fine space validation of the development of the diseases. It is based on Google Maps technology. With a web map a farmer can precisely locate a punctual phenomenon. We validated this tool during a very severe series of storms which strongly damaged the vineyards in May 2009. These data were correlated with those collected during three days in the vineyards with smartphone using GIS application. If wine growers or technicians eventually agree to collect comparison data on diseases, we could have data to calibrate our model.

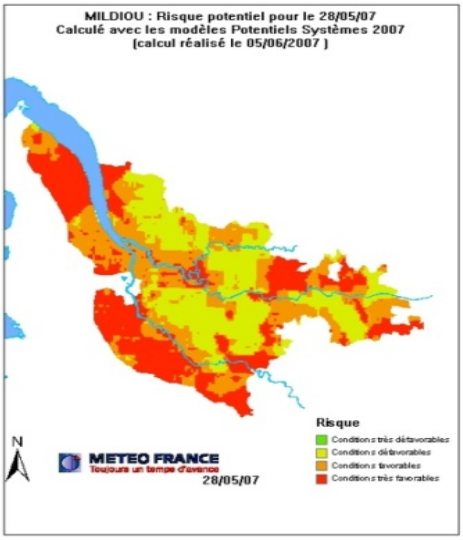

Figure 3: Downy mildew risk map with radar data.

What is new in 2010?

We now start a new program supported by European research funding, which allows us to test the new Hydrix radar technology giving a direct measuring of the amount of rainfall. This radar quantifies the amount of rain for each km² on a 60 km distance so that it can deliver data of about 11 600 gauges. It is also able to quantify rainfall with good precision on a 200 m² scale of a 30 km distance. In addition, we are testing mobile station from Agriscope company to obtain meteo data inside parcels.

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Pesticide dose adjustment to vine foliage for control of downy and powdery mildew in south-eastern French vineyards

M. Claverie[a], S. Devèze[b] , D. Richy[c], C. Girardet[d], A. Davy[e]

[a]Institut Français de la Vigne et du Vin (IFV), Pôle Rhône-Méditerranée, Institut Rhodanien, 2260 Route du Grès, 84100 Orange, France ; [e] IFV, Pôle Aquitaine, 39, rue Michel Montaigne, 33290 Blanquefort, France; [b]Chambre d'Agriculture de Vaucluse, Institut Rhodanien, 2260 Route du Grès, 84100 Orange, France; [c]Chambre d'Agriculture des Bouches-du-Rhône, Avenue Henri Pontier, 13100 Aix-en-Provence; [d]Fredon PACA, 417 Chemin Castellette, Q.Cantarel BP162, 84140 Montfavet, France.

A part of the "Optidose" project. This work belongs to the « Optidose » project carried out at the IFV since 2001. This project is based on the observation that success or failure of a phytosanitary treatment depends on several elements such as pathogenic virulence, vine sensitivity, foliar surface, active ingredient used, quality of spraying. The reference dose per hectare, which in France is the only authorized reference, has been set in order to be efficient while these factors are combined altogether. This is probably not the most common situation, so we must consider whether it is possible to adjust the quantity of pesticides to only the necessary amount. The aim of the "Optidose" project is to propose and then validate a method to adjust the pesticide dose to the situation of the vine that takes into account three factors: leaf area to protect; vine sensitivity; and pathogen virulence (Davy & Heinzlé, 2009). As security margins diminish, adjusting pesticide dose implies a perfect quality of spraying (correctly adjusted sprayers, treatments on both sides of the rows) and a strict respect of the intervals between the applications.

IFV and its local partners have been working on this project in south-eastern Mediterranean vineyards since 2005. Because of the different vineyard conditions in the region (density of planting, cultivars, training systems) we have chosen to focus more on the 'vegetation' part of the program.

Proposing foliar-adjusted doses for south-eastern French vineyards

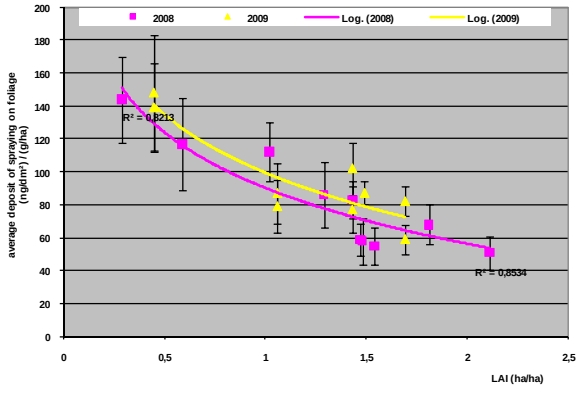
In order to find rules to adjust the dose to the foliage, we chose to work with the variable 'average deposit of spraying par cm² of foliage' (ADS) which is generally patterned after the work of Agroscope Changins-Wadenswil ACW in Switzerland (Viret & Siegfried, 2007, Viret *et al.*, 2008).

Between 2007 and 2009, we followed many sites at different times of the production cycle between May and August under two training systems: double spurred cordon with vertical shoot positioning (VSP) and double spurred cordon with single wire (SW). To make things easier, we chose to work mainly on *Vitis vinifera* cv. Grenache N for its erect bearing canopy. For each measurement, a 20-vine zone was marked on the row in which 10 vines were chosen to work on more specifically. Leaf area and form were described using the Leaf Area Index (LAI) and Tree Row Volume (TRV, average height x width per unit of soil). LAI was obtained by estimating shoot foliar surface of 30 randomly chosen shoots. Shoot surface itself was estimated by counting total leaf number and average leaf surface using the method of measuring the main vein of the leaf (Tregoat *et al.*, 2001) on a 100 leaf sample. Sixteen cm²-filter paper patches were randomly stapled throughout the whole canopy volume in order to sample 1 to 2 per cent of total leaf area (100 to 300 patches per vine stock along with the LAI). Then the vine was treated with a backpack atomizer (Stilh SR 400) using a pesticide-like spray mixture composed of a fluorescent tracer (*Hélios*, Syngenta) suspended in water (550 mg/l). Paper patches were then collected and put into plastic bags in order to analyze the amount of fluorescence (method not presented). The intensity of fluorescence was related to the quantity of tracer found on the paper and thus, per unit of leaf area. Results were expressed in (ng per dm² of leaf area) and normalized per g/ha of tracer used.

The results show that, firstly, total leaf area was quite variable, showing a great diversity of vegetative growth between plots, even at the same period of the year. Measured LAI ranged from 0.25 in May to around 1 at bloom to over 3 on a highly vigorous vine in August. Similarly, during pre-veraison, the same plot showed an LAI of 1 when vines exhibited water stress, to around 2 for non-stressed and vigorous vines. Moreover, for the same value of LAI, the shape of vegetation (i.e. TRV) differed whether the training system was SW or VSP.

Secondly, along with Swiss literature, our results show that the higher the LAI, the lower the normalized value of ASD (Figure 1). Comparing vine plots during May and fully developed plots later in the season, the ADS decreased by a factor 3. At bloom, the ADS was

Figure 1: Variation of the average deposit of spraying per cm² of foliage (ADS) along with leaf area index (LAI) for 2008 and 2009 measurements (bars=standard error).



intermediate, that is, about 2 times the ADS at full development. We observed no difference of ADS between SW and VSP training system.

Validation of the rules for foliage adjustment

These results have been used to propose foliage-adapted doses. As the full dose is expected to be efficient when used at full plant development, i.e. with its lower characterized ADS, we decided to fix the full dose only at the full development stage, and reduced doses for smaller plant development stages. The amount of reduction was determined in proportion of the amount of enhancement of the ADS.

On the basis of these hypotheses, experimental trials have been conducted by the partners 'Chambre d'Agriculture' and 'Fredon PACA' to test the efficacy of powdery and downy mildew control with foliage-adjusted doses. Three treatments were compared: a non treated control ('tnt'), a reference full-dosed treatment ('ref') and a foliar adapted based treatment ('opti'). Trials were developed in 4 repliated experimental plots where sprays were applied to both sides of the vine row using backpack atomizers. The same chemical was used for all treatments : *penconazole* in 2007 and 2008, *tetraconazole* in 2009 for powdery mildew control; *fosetyl-al + mancozebe* for downy mildew control. Starting 2009, organic treatment programs using only copper and sulfur were applied at their own adapted positioning interval and at a full dose reference of 800g copper per ha. Downy mildew is less likely to be found each year under our dry, hot Mediterranean climate, so we chose cultivar 'Carignan' because of its high level of sensitivity to powdery mildew. Springs of 2007 and especially 2008 were favorable to downy mildew resulting in 8 valid trials on downy mildew and 10 on powdery. Disease attack measurements were done at bunch closure for powdery mildew and at berry set and veraison for downy mildew. When necessary, crop weight per vine was recorded as well as maturity analysis. Determination of foliage adjusted dose was done by measurement of TRV and reading the dose in a table.

Foliage adjusted doses led to reductions ranging from 22% up to 65% of the full reference dose, depending on the trial, with a median value of 43%. The frequency and intensity of grape attack for downy (*top*) and powdery (*bottom*) mildew are summarized in Figure 2.

When 'ref' lead to a correct protection, then the 'opti' treatment also gave satisfactory results, although often showing a little more attack but always under an acceptable level. This is true even for important levels of disease attack in 'tnt'. In that case, no harvest loss was recorded (nor difference in maturity) between 'ref' and 'opti' (result not shown).

Some inconclusive cases of diseases control were observed with 'opti', but they were always related to poor results in 'ref' treatments too. Only one trial showed poor control of powdery mildew with the 'opti' treatment although it was correct with 'ref'. This seems to be due to heterogeneity of vegetation, with one vigorous block that was heavily attacked on 'opti' but not in 'ref'. This points out the problem of heterogenic plots (for vegetation or pathogen virulence) and the importance of choosing the most vigorous place too measure TRV. Finally, there were positive results in the organic treatments for which adjusted doses showed similar results to those of the conventional treatments. This treatment will be tested again one more year in 2010 to enhance the number of observations before giving conclusions.

Perspectives: integration of these results in "Optidose" program

Next stage is integration of these results in the "Optidose" program in order to design field trials using the grape grower's own sprayer, treatment program and chemicals. In 2010, most efforts will be spent on field trials in order to test adjusted doses tables under real conditions similar to the way it was done in south-western vineyards. For south-eastern, wide-spaced-row vineyards, an uncertain point is the quality of spray application. In experimental trials shown above, spraying conditions were almost ideal. But pneumatic sprayers or 'atomizers' commonly used in our Mediterranean regions can produce heterogenic sprayings along the side of the vine row treated. This situation must be resolved before "Optidose" tables be considered as validated in our south-eastern vineyards.

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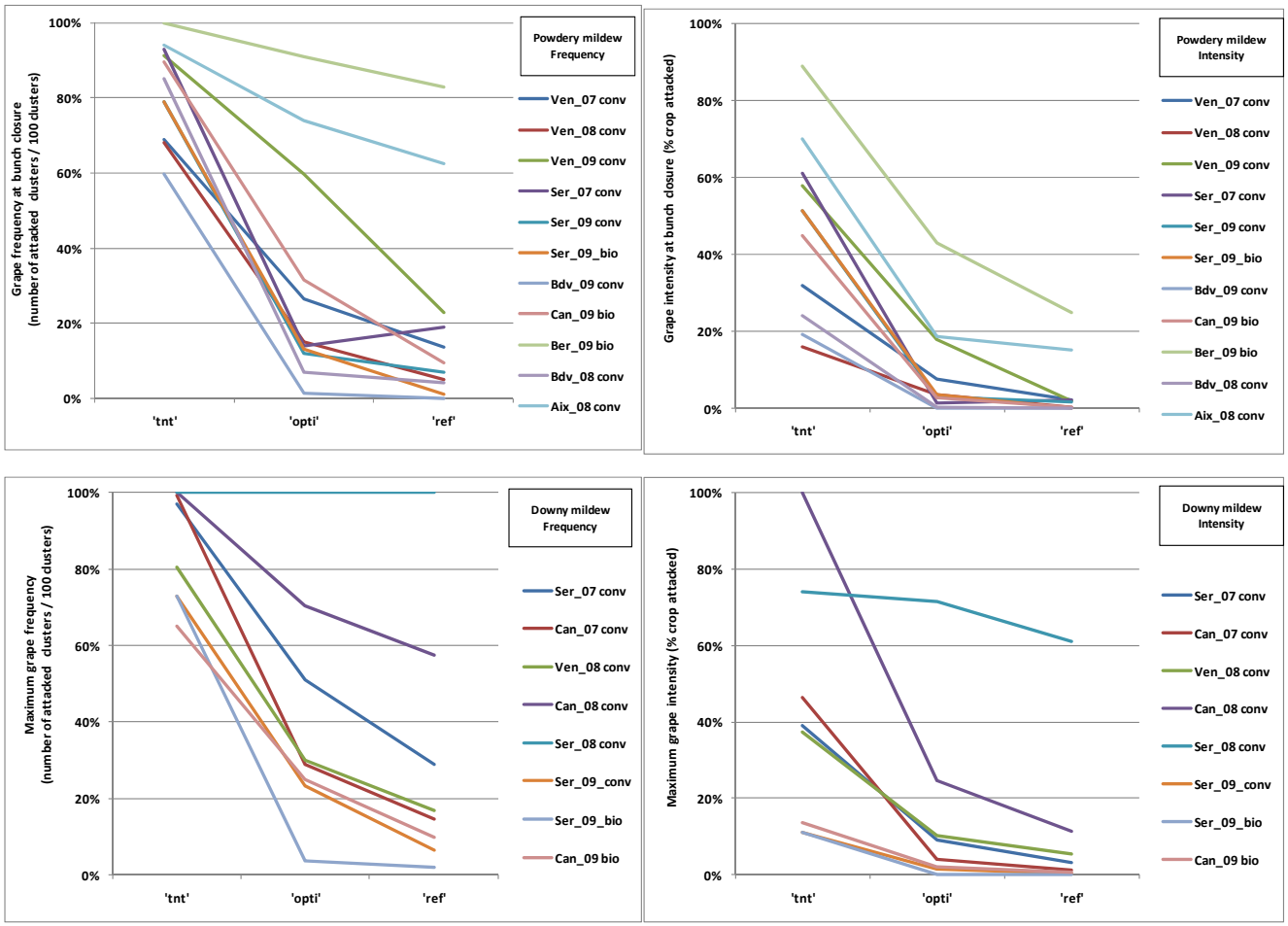
Figure 2: Efficacy of foliage adjusted doses and reference full dose for control of downy (*top*) and powdery (*bottom*) mildew between 2007 and 2009 on experimental plots.



Multiplex®, a potential tool for studying induced resistance on vineyard

N. Aveline[a], A. Riffard[b], MF Corio-Costet[c], S. Cluzet[d], S. Lejealle[e], M. Raynal[a]

[a]Institut Français de la Vigne et du Vin (IFV), [b]ENSA Toulouse, [c]UMR Santé Végétale (INRA), [d]ISVV (GESVAB), [e]Force A. mail : nicolas.aveline@vignevin.com

Plant defense inducers (PDI) have the potential to reduce the use of pesticides in vineyards. For more than fifteen years, many laboratories have studied these compounds. But real gaps persist between encouraging results from the laboratory and their concrete application to the vineyard. A focus is needed at the field scale to define the instructions for using this kind of products in an integrated protection program. Multiplex3® is a hand-held optical sensor, based on real-time and non-contact leaf measurements of fluorescent compounds like poly-phenols, related to plant's defenses. These experimentations are lead by IFV and partners to evaluate the Multiplex® as an effective tool to characterize defenses stimulation of grapevine. To obtain complementary information on assessment of PDI on field trials and to answer to simple questions likes "did grapevine recognize the sprayed elicitor?", assays were made on different cultural conditions (labs, greenhouse and vineyard), with sprays of PDI solutions including reference molecules and commercial products, and different kind of plant material. The main objective was to obtain a real change on any index measured by Multiplex® for a PDI treated leaf vs. control. In these conditions, PDI molecules did not induce changes in curves compared to control. Only UV stress showed typical response, with a significant increase of HCA index. Nevertheless, regarding the simplicity of measurements on field and the data capacity of Multiplex®, further experiments would be done with an improved methodology and new adapted index.

Determination of Genetic Groups and DMI Resistance of *Erysiphe necator* in field samples by a real-time PCR assay

M. C. Dufour[a], S. Fontaine[c] , J. Montarry[b], M. F. Corio-Costet

[a] INRA, UMR Santé Végétale 1065 (INRA-ENITA), BP 81, F-33883 Villenave d'Ornon, France, [b] INRA-Avignon, UR407 Pathologie Végétale, F-84143 Montfavet, France, [c] AFSSA Lyon, Unité Résistance aux Produits Phytosanitaires, 31 Avenue Tony Garnier,69 364 LYON Cedex 07

Since the introduction of the causal agent of grapevine powdery mildew (*Erysiphe necator*) to Europe, pesticides have been required to suppress the disease. Nevertheless, wine growers sometimes encounter important problems in the management of epidemics with the appearance of fungicide resistance in pathogen populations in vineyard. Such is the case with the demethylation inhibitors (DMI), which inhibit the sterol biosynthesis in fungi. Because of the biological characteristics of powdery mildew, resistance to the DMIs appeared in the late 80s (Steva *et al.*, 1989).

Erysiphe necator, an obligate biotrophic parasite also presents the distinguishing feature of occurring in different vineyards in two distinct genetic groups called populations A and B (Corio-Costet, 2007). The distribution and the epidemiological significance of the two groups are poorly understood. Based on the over-wintering modes, groups A and B could be responsible for the primary inoculum arising from sexual reproduction and/or asexual wintering of mycelium in dormant buds (see in 2). In spring, at the beginning of grapevine growth, symptoms appear in the flagshoot form (asexual) or sparse plots arising from ascospore projection (sexual). Several studies have reported the presence of the two groups with variable temporal distribution between the beginning and the end of the growing season (Corio-Costet, 2007; Corio-Costet *et al.*, 2000, Miazzi *et al.*, 2008; Montarry *et al.*, 2009). How the selection pressure of fungicides could acts on the two genetic groups is not known? Work undertaken in our laboratory also showed that the group A isolates from France are more sensitive to fungicides than isolates belonging to genetic group B (Corio-Costet, 2007).

Determination of fungicide sensitivity in pathogen population and their diversity and adaptive potential is the first important step in the management of fungicide resistance. The most commonly used test for determining DMI sensitivities of *E. necator* isolates is based on a biological test with mycelium growth inhibition that is very fastidious, in particular with an obligate parasite like *E. necator*. It is also possible to use a nested-PCR method or a PCR-RFLP method to detect DMI resistance, based on the detection of single nucleotide polymorphism (SNP) in *CYP51* gene at codon Y136F. However, this method is not quantitative and requires many individual samples from a plot to obtain a good idea of the DMI resistance level (Montarry *et al.*, 2009; Delye *et al.*, 1999).

For genetic group determination, a qualitative test is possible for screening field population by nested PCR or PCR-RFLP methods (Delye *et al.*, 1999; Peros *et al.*, 2006), but quantitative tests need numerous samples with individual genotype determination. The method developed in this study was initially designed as an alternative to traditional qualitative and quantitative tests. It was designed to quantify the proportion of a specific allele genotype (A or B) in a population, with the possibility of pooled samples. Several genotypic quantifications can be specified on the same sample or population, such as genetic group A or B, DMI resistance and QoI resistance frequencies. These quantitative methods could be used for dynamic population studies to measure the genetic group evolution of *E. necator* throughout the growing season, and to monitor resistance to DMI and QoI fungicides in vineyard. The objective of this study was thus to establish an inventory of aspects of resistance such as the DMIs and for of more recent features as the QoIs in the French vineyard, associated with the distribution of the genetic groups A and B.

Material and Methods

Strains used for Q-PCR were from our laboratory's collection (Table 1).

Table 1. Characteristics of *E. necator* isolates used in this study

Origin	N	Genotype	Fungicide status
Languedoc, France	5	A	DMI sensitive
Aquitaine, France	5	B	DMI sensitive
Bengalore, India	1	B	DMI resistant
Eger, Hungaria	2	B	QoI resistant *

* All strains were QoI sensitive except the two isolates from Hungary.

The various isolates were multiplied on Cabernet-Sauvignon vine leaves in Petri dishes as previously described Corio-Costet, 2007; Delye *et al.*, 1999). The inoculations were done between 1000 in 1500 conidia per cm^2 of leaves and incubated for 12 days in 22°C in a climatic chamber.

Field sapling was conducted in French vineyards from May to the beginning of September in 2008 and from the end of June to the end of September in 2009 in 6 grapevine production regions. A total of 689 grapevine lesions were collected in 2008 and 650 in 2009. For each sample, 10-15 lesions from leaves (16 mm of diameter) were pooled (n = 52 in 2008 and 51 in 2009). The mycelium of *E. necator* on grape berries was scraped from 15 bunches, pooled in Eppendorf tubes and stored at -20°C. DNA was isolated from frozen tissue samples or directly from lesions as described previously (Amrani *et al.*, 2006). PCR-RFLP method was described by Montarry *et al.* (Montarry *et al.*, 2008). Real time PCR protocol is described in Dufour *et al.* (Dufour *et al.*, 2010a and b).

Results and Discussion

Primers were designed from specific SNP on CYP 51 gene of *E. necator* at codon G37A and Y136F for genetic group A and DMI resistance respectively (Dufour *et al.*, 2010b). They were tested on various dilutions of DNA of *E necator* isolates, belonging either to group A or being resistant in fungicides, to obtain calibration curves. From these curves the total quantity of DNA and the DNA possessing an interested allele were quantified, allowing us to determine quickly the frequencies of DNA belonging to group A or possessing F136Y.

Standard calibration curves were constructed with a range of allele-specific frequencies (from 0.36 to 100 % of B *vs* A genetic group, or sensitive *vs* resistant DMI using the specific DNAs for the respective alleles (figure 1). The experiment was performed on three separate SNPs. The specific allele was expressed as relative quantity using an

allele (EN for *Erysiphe necator*) common to two genetic group content in the same sample, as an internal calibrator.

The difference between the two PCR reactions (ΔCq) was

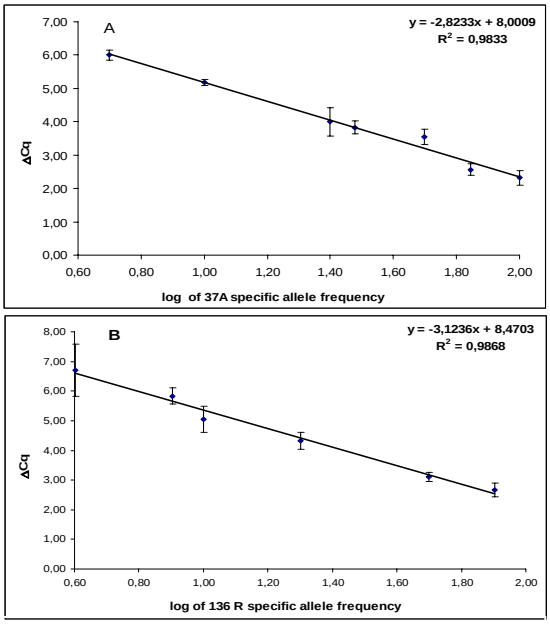


Figure 1: Linear relationship between frequency of specific alleles and ΔCq values determined with real time PCR assays. A: allele specific to group A (q-EN-37A), B: specific allele of DMI resistance (q-EN-136).

used to calculate the allele frequency according to the direct correlation between ΔCq (ΔCq = $Cq_{EN} - Cq_{allele\ specific}$) and the decimal logarithm of allele-specific frequency. The logarithm of the known allele frequency was plotted as a function of the ΔCq measured between the two PCR reactions and a trend line was estimated. To test the linearity of SNP allele quantification, ΔCq values were used for linear regression analysis.

The primers developed were able to amplify DNA of *E. necator* identified as group A, or as DMI resistant. Q-PCR analysis of group A and B mixtures showed a better sensibility than the PCR-RFLP method. With q-PCR assays it is possible to detect and quantify group A or DMI resistant isolates, with a quantification limit of 2.40 and 2.85% and a detection limit of 0.72 and 0.85%, respectively. In the case of *E. necator*, an obligate parasite, to be able to identify and quantify pooled samples directly from field is a real step forward for monitoring populations in vineyards.

Frequency of Group A allele in field samples

The A/B ratios observed for the field samples in 2008 (Table 2) and 2009 (Table 3) were variable, with an average of 18.44 and 7.44% of group A in the vineyard, respectively. Thus, group B was prevalent in French vineyards. The ratios of group A to group B varied from 0 to 100% depending on the locality. In 2008, only the samples from Languedoc-Roussillon-2 collected at the beginning of the growing season were significantly different from the samples from other areas since they showed 75% of group A. On the contrary, the samples from Languedoc-Roussillon-1 collected two months later exhibited only 11% of group A. In 2009, this locality presented only 26 % of group A corresponding to samples collected at the middle of growth season. Although the samples did not come from the same plots, we suggest as described previously (Corio-Costet *et al.*, 2000; Montarry *et al.*, 2009), that group A was more present at the

Table 2: Distribution of Genetic group A specific allele and DMI resistant allele of *E. necator* in different French vineyards in 2008.

Region	N	Mean percentage of G37A allele (Group A) ± SEM	Mean percentage of Y136F allele (DMI resistant)± SEM
Aquitaine	6	0 ± 0	0 ± 0
Bourgogne	7	1 ± 0.80	5.51 ± 3.49
Champagne-Ardennes	6	0.96 ± 0.96	0 ± 0
Languedoc-Roussilon -1	6	11.30 ± 7.20	0 ± 0
Languedoc –Roussillon -2	10	75.13 ± 11.84	1.43 ± 1
Midi- Pyrénées-1	10	10.25 ± 6.84	0.26 ± 0.18
Midi-Pyrénées-2	5	1.16 ± 1.16	18.77 ± 18.82
PACA	2	9.39 ± 6.25	3.85 ± 1.38
National average		18.44 ± 4.73	3.02 ± 1.86

beginning of the growing season, and that later in the growing season it tended to decrease in the vineyard and be replaced by group B. Furthermore, group A was more present on leaves (28% in 2008 and 18 % in 2009) than on grape berries (8% in 2008 and 3 % in 2009) (data not shown) suggesting that group A isolates are less aggressive on berries than group B, as reported for other genetic groups of fungus like *Botrytis cinerea* .

Table 3: Distribution of Genetic group A specific allele and DMI resistant allele of *E. necator* in different French vineyards in 2009.

Region	N	Mean percentage of G37A allele (Group A) ± SEM	Mean percentage of Y136F allele (DMI resistant)± SEM
Aquitaine	3	0 ± 0	12.26 ± 12.26
Bourgogne	8	1.82 ± 0.90	1.13 ± 1.12
Champagne-Ardennes	2	6.31 ± 6.31	37.27 ± 31.40
Languedoc-Roussilon 2	12	26.09 ± 11.66	12.75 ± 8.21
Midi-Pyrénées-2	22	0.70 ± 0.30	35.15 ± 9.40
PACA	4	8.86 ± 8.86	13.67 ± 11.13
National average		7.44 ± 5.00	21.85 ± 4.90

This result was in agreement with previous studies describing genetic group A as less aggressive than group B or group A being less present on grape berries than group B (Montarry *et al.*, 2009) and reinforce the idea that the two groups could have different ecological requirements. In previous studies, group A seemed to be more present in Mediterranean areas than in other areas. The q-PCR assays show here that the areas bordering the Mediterranean Sea exhibit a significantly higher frequency of group A (46% in 2008 and 30 % in 2009) than other areas (average of 3% in 2008 and 11 % in 2009). This result suggests the possibility of geographical variation in the population structure of *E. necator* due to climatic environment, cultural practices, and/or sensitivity of cultivars to grapevine powdery mildew cultivated in this area.

Frequency of DMI-resistant allele and QoI-resistant allele in field population of E. necator.

The DMI resistant allele Y136F was present at a national level of 3% in 2008 and 22 % in 2009. This strengthens the idea that the DMI resistance of grapevine powdery mildew in French vineyards was limited but has increased. Thus, 10 samples in 2008 and 12 in 2009 (19% and 23% respectively) were detected as resistant, with only two

localities exhibiting more than 20% of Y136F allele in 2008. One sample from the Midi-Pyrénées-2 area exhibited a high frequency of DMI resistant allele (93%) and simultaneously a high frequency of QoI resistant allele (30%, data not shown). The powdery mildew population of these plots of land, belonging to 100% to genetic group B, and was doubling resistant to DMI and QoI fungicides. In 2009, twelve samples were detected as being DMI resistant, with 4 localities exhibiting more than 20% of the Y136F allele. It would be interesting to follow this plot of land for several years and to modify the treatment program which systematically contains four DMI treatments per growing season. This second year of study showed that there is a little evolution in France for DMI resistance based on the Y136F. The presence of QoI resistant allele at the frequency of 31% in one locality in 2008 and 100 % in five localities in 2009 suggests that fungicide pressure could select double resistance in *E. necator* populations. To date no report has shown double fungicide resistance in grapevine powdery mildew, which is considered as a pathogen with a slight adaptation capacity.

Conclusion

The different tools developed here represent a real benefit for *E. necator* management. The possibility of combining quantification of resistant alleles with the presence of genetic groups A and B could throw light on the role of the genetic group in the progress of the resistance depending on the biologic characteristics of the different groups. Indeed, genetic, group A, which is more present in southern French vineyards over-wintered in dormant buds obviously without sexual reproduction in France and group A isolates were significantly more sensitive to different fungicides than group B isolates. Combining the possibility to quantify the ratio of the two groups in different vineyard areas, with the frequency of different resistant alleles could also lead to improving the understanding of these two genetic groups and to enhancing pest management.

These new diagnostic methods have numerous advantages: i) the method is not based on expensive fluorescent labeled primers or probes, ii) it is an approved assay that requires no post PCR processing (RFLP or nested–PCR), iii) it promises to be time-saving and precise enough to allow detection of weak genetic associations, iv) it is possible to estimate genetic variability on a large scale owing to the possibility to work with pooled samples *vs* individual symptoms. This study is the first that makes it possible to obtain a wide panorama of *E. necator* diversity in French vineyards more easily than nested-PCR or PCR-RFLP methods on individual samples. It would now be possible to analyze more precisely the distribution of the different genetic groups and test different hypotheses. Moreover, knowing what the resistance status of a plot to fungicides is represents an advantage for choosing the appropriate treatment strategy. This assay could prove useful in routine monitoring of pest management in vineyards, which may in turn provide timely warning for winegrowers.

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Differences in incidence and severity of powdery mildew and downy mildew among cold climate wine grape cultivars and table grape cultivars in 2009.

L.P. Berkett, T.L. Bradshaw, S.L. Kingsley-Richards, M.L. Cromwell

Department of Plant and Soil Science, University of Vermont, Burlington, Vermont 05405, USA

Cold climate wine grape production is a rapidly emerging "new" crop in the diversification of agriculture in Vermont and other northeastern states offering significant value-added and agri-tourism economic opportunities. In the past, commercial wine grape production was not recommended in the colder regions of the USA because of problems with winter survival of the vines. However, cold climate wine grape cultivars developed by the University of Minnesota breeding program and Elmer Swenson, a private breeder, are now available commercially. These wine grape cultivars survive -34^{0}C to -37^{0}C winter temperatures (University of Minnesota, 2008) and are being planted in Vermont on newly created farms or as an alternative crop on existing farms.

Since high quality fruit is the basis for quality wine production, cultivar selection is a critical decision for a grape grower which will impact the vineyard's competitiveness and profitability, and the success or failure of the vineyard. A key challenge to this young industry is the selection of wine grape cultivars which will survive Vermont's cold winters and consistently produce high quality fruit within cool, relatively short growing seasons. More than 75% of Vermont has an average minimum cold temperature below -29^{0}C and, in the remainder of the state, the average minimum cold temperature is between -26^{0}C to -29^{0}C (Perry, 2003). In Burlington, VT, the lowest winter temperature can fall between -28^{0}C to –34^{0}C (NOAA, 2008*)*. Over the last ten years, the average growing degree days (base 10^{0}C) was 2384.

Another key challenge is to manage diseases effectively in the most environmentally- and economically-sustainable manner. Fortunately, in addition to cold-hardiness, these cold climate interspecific hybrids were bred for disease resistance (University of Minnesota, 2010) and potentially may require less overall fungicide use to produce high quality fruit. However, little research has been conducted to determine their relative disease susceptibility and fungicide requirements.

The purpose of this research was to compare disease incidence and severity among eight wine grape cultivars from various sources, including University of Minnesota and Swenson hybrids, planted in the University of Vermont experimental vineyard and managed using an integrated pest management (IPM) approach (Berkett, 2009). Eight table grape cultivars planted in the vineyard were also evaluated.

Materials and Methods

The research was conducted in a vineyard at the University of Vermont Horticulture Research Center, South Burlington, Vermont, USA [Lat: N 44.4308; Lon: W 73.2042; Elev: 71 meters]. The vineyard was planted in 2007 with eight wine and eight table grape cultivars considered to be the most "promising" based on the experience and insights of current Vermont grape growers and from published information. A randomized complete block experimental design of six blocks with four-vine plots of each cultivar per block was used to plant the following eight wine grape cultivars: Frontenac, LaCrescent, St. Croix, Marquette, Prairie Star, Corot Noir, Vignoles, and Traminette. The first five cultivars listed are considered cold climate cultivars whereas Corot Noir, Vignoles and Traminette are more cool climate cultivars but were included for comparison. The vines were planted 1.8 m apart within each row and 3.0 m between rows. The vines are trained to a high-wire cordon system. The soil is a well-drained Windsor loamy sand; the vineyard has drip irrigation. This vineyard is part of the multi-state research project (NE-1020) in the USA to evaluate wine grape cultivars and clones (USDA, 2010). It represents the coldest winter and coolest growing season conditions of any of the NE-1020 sites in the eastern USA. Since growers in the region are also interested in table grapes, the following table grape cultivars were included in the vineyard in two-vine plots with six replications per cultivar planted randomly around the perimeter of the vineyard: Beta, Concord, Einset Seedless, Mars, Reliance, Somerset Seedless, Swenson Red, and Vanessa. Of these cultivars, Beta, Somerset Seedless and Swenson Red were developed by the University of Minnesota breeding program and/or by Elmer Swenson (Smiley *et al.*, 2008)

During the growing season, environmental conditions were monitored with an on-site Davis Vantage Pro Wireless Weather Station (Davis Instruments Corp., 3465 Diablo Ave., Hayward, California 94545 USA). Environmental conditions and information on the critical periods to manage grape diseases were integrated to determine the need to apply fungicides during the growing season. Selection of specific fungicides was based on efficacy, spectrum of activity, availability of 'reduced-risk' alternatives, and resistance management considerations. A total of five fungicide applications were made in 2009. On 26 May, mancozeb (Dithane DF Rain Shield, Dow AgroSciences LLC, Indianapolis, IN) was applied at 4.48 kg/ha. Mancozeb and myclobutanil (Rally, Dow AgroSciences LLC, Indianapolis, IN) were applied on 12 June at 4.48 and 0.35 kg/ha, respectively. Two captan applications (Captan 80 WDG, Drexel Chemical Co., Memphis, TN) were made on 25 and 30 June at a rate of 2.8 kg/ha. The first captan spray included myclobutanil (0.35 kg/ha), while on 30 June, kresoxim-methyl (Sovran, BASF Corp, Research Triangle, NC) at 0.35 kg/ha was applied with the captan. On 10 July, kresoxym-methyl was applied alone at 0.35 kg/ha. All applications were applied to all vines in the vineyard with a Rears Pul-Blast 300 Airblast Sprayer (Rears Manufacturing, Inc., Eugene, OR) calibrated to deliver 748 liters per hectare using only hydraulic pressure (1.035 MPa) since utilization of air assist would result in significant spray drift. The vines also

received two standard insecticide sprays during the 2009 growing season.

Disease incidence and severity on fruit clusters of wine grape and table grapes were determined by visually assessing ten randomly selected clusters per wine grape plot and five table grape clusters per plot on 2-4 September. Foliage disease incidence and severity on wine grape plots were determined by examining 20 randomly selected leaves per plot collected on 16 September. The Horsfall-Barrett scale was used to rate disease severity (area infected) on clusters and wine grape foliage. For the table grape cultivars, a more general assessment of the foliage was conducted; each plot was visually scanned in the vineyard for the presence of foliar disease and if observed, an overall subjective rating was given for the plot: 0= no symptoms observed; 1= low level of symptoms observed; 2= moderate level observed; 3= severe level observed. All proportional data were transformed using the arcsin sqrt transformation before statistical analysis.

Results

The 2009 growing season can be characterized as unusually wet and cool. Monthly rainfall amounts were 13.3 cm, 13.3 cm, 11.7 cm, and 5.9 cm for May, June, July and August, respectively, with a total of 57 days with at least 0.025 cm of rain. Consequently, more fungicides were applied because of concern about residual coverage for the various diseases that affect grapes in the region in addition to powdery mildew, *Erisiphe necato*r, and downy mildew, *Plasmopara viticola* (i.e., black rot, *Guignardia bidwellii;* Phomopsis cane and leaf spot, *Phomopsis viticola;* angular leaf scorch, *Pseudopezicula tetrasporal* and anthracnose, *Elsinoe ampelina*).

Percent incidence and severity for powdery and downy mildew on the various wine grape cultivars are summarized in Table 1. Foliar powdery mildew was observed on all wine grape cultivars with Prairie Star, Frontenac, and LaCrescent having the highest incidence (60.83%, 51.67%, and 41.67%, respectively). However, no powdery mildew was observed on fruit clusters of any wine grape cultivar except Corot Noir where a small percentage of clusters (1.67%) was observed to be infected.

Downy mildew foliar symptoms were observed only on Vignoles and Corot Noir where there was a high incidence of disease (93.33% and 79.17%, respectively). No symptoms were observed on the fruit of any of the wine grape cultivars.

All of the table grape cultivars except Mars exhibited foliar powdery mildew but no powdery mildew was observed on the fruit clusters of any cultivar (Table 2). Downy mildew was only observed on the foliage of one plot of Einset Seedless; no downy mildew symptoms were observed on fruit clusters of any table grape cultivar (Table 2).

Summary

Although all vines were treated the same, there were differences in incidence and severity among cultivars for both powdery mildew and downy mildew which may reflect their relative susceptibility to disease. On average, eight fungicide applications are typical for *Vinifera* and French hybrid grapes in the region (Weigle *et al.*, 2003). Given the 2009 disease management program which included a total of five fungicide applications to address all the key diseases for the site, it is interesting to note that no downy mildew was observed on the fruit of any cultivar and symptoms were present only on the foliage of two wine grape cultivars not considered to be cold climate cultivars, and on one table grape cultivar. In contrast, powdery mildew was more prevalent on foliage across both the wine and table grape cultivars. More research is needed to determine the innate disease resistance/susceptibility of these cultivars and how best to incorporate this knowledge into effective disease management programs that address economic, health, and environmental concerns.

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Table 1: Comparison of disease incidence and severity of powdery mildew and downy mildew among eight wine grape cultivars in 2009.

	Wine Grapes							
	Powdery mildew				Downy mildew			
	Leaves*		Clusters*		Leaves*		Clusters*	
Cultivar	% Incidence	% Area infected	% Incidence	% Area infected	% Incidence	% Area infected	% Incidence	% Area infected
Corot Noir	21.67 bc	1.61 bc	1.67	0. 04	79.17 b	9.91 a	0.00	0.00
Frontenac	51.67 a	2.51 ab	0.00	0.00	0.00 c	0.00 b	0.00	0.00
LaCrescent	41.67 ab	1.77 abc	0.00	0.00	0.00 c	0.00 b	0.00	0.00
Marquette	10.00 cd	0.39 cd	0.00	0.00	0.00 c	0.00 b	0.00	0.00
Prairie Star	60.83 a	4.78 a	0.00	0.00	0.00 c	0.00 b	0.00	0.00
St. Croix	10.83 cd	0.89 bcd	0.00	0.00	0.00 c	0.00 b	0.00	0.00
Traminette	1.67 d	0.04 d	0.00	0.00	0.00 c	0.00 b	0.00	0.00
Vignoles	2.50 d	0.12 d	0.00	0.00	93.33 a	12.44 a	0.00	0.00

Table 2: Comparison of disease incidence and severity of powdery mildew and downy mildew among table grape cultivars in 2009.

	Table Grapes							
	Powdery mildew				Downy mildew			
	Leaves*		Clusters**		Leaves*		Clusters**	
Cultivar	% Incidence	Relative Rating	% Incidence	% Area infected	% Incidence	Relative Rating	% Incidence	% Area infected
Beta	83.33 a	0.8333 bc	0.00	0.00	0.00	0.0000	0.00	0.00
Concord	50.00 ab	0.5000 c	0.00	0.00	0.00	0.0000	0.00	0.00
Einset Seedless	100.00 a	2.0000 a	0.00	0.00	16.67	0.1667	0.00	0.00
Mars	0.00 b	0.0000 c	0.00	0.00	0.00	0.0000	0.00	0.00
Reliance	100.00 a	2.1667 a	0.00	0.00	0.00	0.0000	0.00	0.00
Somerset Seedless	100.00 a	2.5000 a	0.00	0.00	0.00	0.0000	0.00	0.00
Swenson Red	16.67 b	0.1667 c	0.00	0.00	0.00	0.0000	0.00	0.00
Vanessa	100.00 a	1.6667 ab	0.00	0.00	0.00	0.0000	0.00	0.00

*Values represent the mean percentage of the 6 replicate 2-vine plots per cultivar on which any foliar symptoms were observed. Disease severity was rated per plot using a subjective, relative rating system of 0 = no foliar symptoms observed; 1= low level of symptoms observed; 2= moderate level observed; 3= severe level observed. Means followed by the same letters within columns are not significantly different according to Tukey's Studentized Range (HSD) Test ($P \leq 0.05$).

**Values represent the mean of 6 replicate 2-vine plots per cultivar from 5 clusters per plot. Disease severity (area infected) was rated using the Horsfall-Barrett scale and converted to percentages using the Elanco conversion tables. Data were transformed using the arcsin sqrt transformation before statistical analysis. Means followed by the same letters within columns are not significantly different according to Tukey's Studentized Range (HSD) Test ($P \leq 0.05$).

VitiMeteo-Plasmopara forecasting tool as part of www.agrometeo.ch interactive platform

O. Viret[a], P.-H. Dubuis[a], A.-L. Fabre[a], B. Bloesch[a], W. Siegfried[b], A. Naef[b], M. Hubert[b], G. Bleyer[c], H.-H. Kassemeyer[c], M. Breuer[c], R. Krause[d]

[a]Agroscope Changins-Wädenswil ACW, Route de Duillier, CH-1260 Nyon, [b]Agroscope Changins-Wädenswil ACW, Schloss, CH-8820 Wädenswil, [c]Staatliches WeinbauinstitutFreiburg, 79100 Freiburg, Germany, [d]GEOsens Ingenieurpartnerschaft, 79285 Ebringen, Germany

The implementation of disease forecasting models to control the main fungal disease of vineyard according to their epidemiology is a central element of integrated pest management. The use of decision support systems (DSS) is increasing in importance among advisers and growers. Agrometeo is a free available interactive platform (www.agrometeo.ch) containing a broad range of tools for agriculture in Switzerland, including weather data, modules for grapevine, fruit orchards and field crop.
The grapevine module contains forecasting models for downy mildew and grape berry moths; leaf area adapted spraying calculator, growth development model, Swiss pesticides index, and descriptions of the main grapevine's diseases and pests. Furthermore, a forecasting model for powdery mildew is in validation and will be soon available on internet. The forecasting modules use data from a weather station network covering the whole viticulture area of Switzerland. The setup of a national network of weather data at the microclimatic level started in 2000 and Internet display of the values was freely accessible to the growers since 2002. To date 150 weather stations (Campbell CR10X, Campbell CR 1000, Lufft HP-100, Lufft Opus) are measuring temperature (°C), relative humidity (%), leaf wetness duration (h) and rainfall (mm) under field conditions, covering the whole country. Ten minutes data are sent via GSM, two times a day.

Forecast for downy mildew. The VitiMeteo-Plasmopara model (Bleyer et al., 2008a, 2009; Viret et al., 2005) developed by the Grapevine Research Institute of Freiburg (Germany), Agroscope ACW (Switzerland), and programmed by the company GEOsens (Germany), simulate the main development steps of the epidemiology of *Plasmopara viticola* (Bleyer et al., 2008b; Viret et al., 2007). The software generates graphics and tables freely available for the growers on the Internet. Results are presented as summarized tables and graphs for each region with the possibility to access to detailed tables containing all relevant data from the first of January on. The predicted downy mildew risk for the next five days, based on weather forecast from meteoblue (Basel, Switzerland) appears greyish on the tables and graphs. Parameters included in the model can be adjusted after experimental values by the experts. An observation laboratory under field conditions has been build up for the validation of Vitimeteo-Plasmopara. Control sensitive cv. Pinot noir and Gamay vines are grown above a stock of downy mildew infected leaves replaced every autumn. To follow oospores maturation, leaf pieces of approx. 5 mm^2 are selected under the binocular (to ensure the presence of oospores) and placed over the winter in 50 ml Falcon tubes deep in the soil. In spring, at regular intervals, the leaf pieces are transferred in Petri dishes at 100% rH and the emergence of primary sporangia is counted every day under the binocular. Oospores are considered to be mature, when germination occurs within 24 h. From that date on, trap-plants (cuttings with 6-7 unfolded leaves cv. Chasselas grown during winter in greenhouse) are placed before each rainfall over a stock of infected leaves and incubated in the greenhouse after the rain, to check for oil spots (100% rH, darkness, 20°C). Validation of the model by comparing real epidemic data from field observations and VitiMeteo-Plasmopara infection prediction shows a good correspondence. In general, the use of VitiMeteo-Plasmopara for diseases and pests management is evaluated positively by extension services and vine-growers. Under the Swiss climatic conditions with locally high downy mildew pressure, VitiMeteo-Plasmopara is a precious tool for a more precise control of the disease. Growers using it are spraying more in accordance with the epidemic and in dry years they can objectively delay the first spray and decide to enlarge spray intervals, reducing so the number of sprays.

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Reduction of pesticide doses with the use of softened water, additives and alternatives products

E. Serrano, V. Viguès, P. Saccharin

Institut Français de la Vigne et du Vin (IFV), Pôle Sud Ouest V'Innopôle – 81310 Lisle/Tarn – France, eric.serrano@vignevin.com

The policy of today in France and Europe is tending towards decreasing the use of pesticides. One of a way is to reduce the doses of the pesticides during the applications. Different ways are been suggested to the growers.

The objective of our work is to estimate the efficiency of some of them: use of processes modifying the physico-chemical characteristics of the water, addition of additives in spray and the use of alternatives products (phosphites).

Reduction of doses and softened water or additives: Synthesis of 5 trial years

The water softener used in these trials consists of decreasing the hardness of the water by a passage on an exchanging resin of ions. The ions calcium and magnesium are eliminated and replaced by ions sodium.
Two additives were tested over the years: LI 700 (soya lecithins) and Héliosol (terpene alcohols).

Conditions of experiment. The tests were carried out in natural conditions, for 5 years on a plot of Duras and Mauzac B. situated in AOP GAILLAC.
Four modalities were compared. Each of them contain 3 repetitions of 12 vines:

- Reference : full dose with tap water
- ½ dose : half dose with tap water
- ½ dose + soft water : half dose with water softener
- ½ dose + additives : half dose with the addition of additives
- Untreated: untreated plot.

Treatments were carried out with a manual pneumatic atomizer (Sthil SR 400) in 100L / ha.
Several notations (on 100 leaves and 100 grapes) were carried out during the season. The last one (at the veraison) was the most revealing of the efficiency of a program of treatments. The softened water was controlled by checking the hardness of the water before and after passage on resins.

Results of softened water. According to the year, the tap water used appears between 10 and 23 times harder than the soft water: the hardness of the tap water varied between 14 and 42,3°F while the hardness of the soft water varied between 0,6 and 7,9°F.

Figure 1 shows the efficiency of the different treatment strategies on leaves.
The reference "full dose" showed an average efficiency of 94% during the five years of trials and the "half dose" about 83% when disease is highly expressed in the vineyard.

The dose reduction leads to a loss of efficiency, but this decrease is similar as the water is soft or not.
Over five vintages and whatever the level of pressure of downy mildew, the use of softened water does not improve the efficiency of the half dose of pesticides.

Results of additives. Over five vintages and whatever the level of pressure (downy mildew), the use of additives did not improve the efficiency of pesticide treatments.

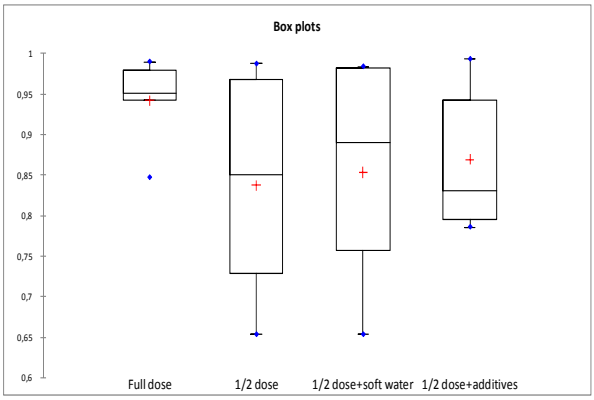

Figure 1: Average efficiency against downy mildew relative to the frequency of attack on untreated plot during five years of testing.

Dose reduction and alternatives: trials of phosphites

Molot (2007) and Aveline (2008) showed, after several field trials, that the efficiency of phosphites, sprayed without others fungicides is low and unacceptable but seems to be interesting when phosphites are associated.
In 2009, we tested the efficiency of phosphites associated with a low dose of folpel (1000g) against downy mildew.

Conditions of experiment. The tests are conducted under controlled conditions (artificial contamination, brumisation) on a plot of Mauzac B. situated in AOP Gaillac (France).
7 modalities were compared:
• Sémafort associated with 1000g folpel
• Trafos associated with 1000g folpel
• PK2 associated with 1000g folpel
• Nutriphite associated with 1000g folpel
• Labifito associated 1000g folpel
• Reference : Mikal (fosetyl Al with folpel)
• 1000g folpel

Each modality has 3 blocks of 6 vines. An untreated plot has been implemented.

Brumisation cycles were made in order to maintain a high pressure epidemic. The rate of treatment is 10 days. The treatments are performed using a manual pneumatic sprayer (Stihl SR 400).

Results

The application of phosphites as a supplement to a classic pesticide product as folpel improves the efficiency of this product. In the conditions of our trial, the efficiency of folpel is less than 50%.

The improvement is not the same according to the phosphite.

With the majority of our notations, the fosetyl is the substance which best optimizes the efficiency of the folpel (figure 2).

In this optics of use, Trafos and Sémafort seem the most interesting after fosetyl, in particular at the beginning of the season.

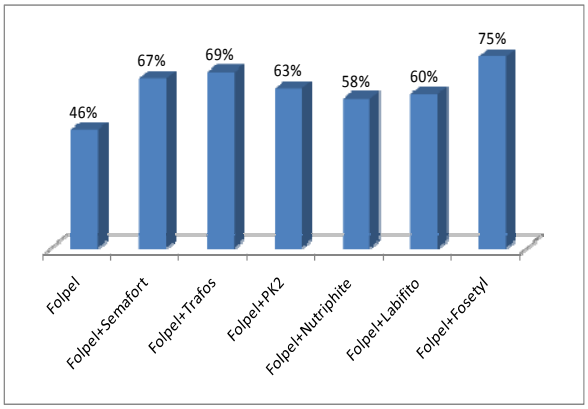

Figure 2: Efficiency against downy mildew relative to the frequency on leaves of attack on untreated plot

Conclusion

Reduction of dose and preservation of an acceptable efficiency are possible. Davy (2007) showed that an adaptation of the fungicide dose applied based on the plant surface, the diseases pressure and the development stage was possible.

But in this optics, the use of softened water or additives is not relevant. These methods bring no improvement in terms of efficiency.

The application of phosphites as a supplement to a classic pesticide product as folpel, but used at a low dose, is a way to improve the efficiency of the product.

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Evaluation of organic fungicides for control of downy and powdery mildew of grapes

A. M. C. Schilder, J. M. Gillett, and R. W. Sysak

Department of Plant Pathology, Michigan State University, East Lansing, Michigan, USA.

Grapes are also an important fruit crop in Michigan grown on almost 5,800 ha (Kleweno and Matthews, 2009). Most of the production area is devoted to juice grapes with wine grapes produced on about 1,000 ha. Fungal diseases represent a continuous challenge because of the humid climate. Due to the limited number of fungicide products allowed in organic production (Wise et al., 2009), these diseases are difficult to control. Organic grape growers tend to have mostly sulfur, copper, and lime sulfur available for managing diseases. Unfortunately, a number of grape cultivars are sensitive to sulfur, copper or both, which precludes the use of these products on these varieties. In addition, sulfur may reduce beneficial mite populations, and copper may be detrimental to soil fauna. The objective of this study was to evaluate the efficacy of organic fungicides listed by OMRI (the Organic Materials Review Institute) for control of powdery and downy mildew in grapes.

The experiment was conducted in a mature *Vitis labrusca* 'Niagara' vineyard at the Trevor Nichols Research Complex in Fennville, Michigan, USA in 2008 and 2009. The following fungicides were evaluated at recommended rates in 2008: Serenade Max (*Bacillus subtilis*), Sonata (*Bacillus pumilis),* JMS Stylet Oil (paraffinic oil), Neu 1160 Vegetable Oil (canola oil), Sporan (rosemary oil, clove oil, thyme oil, wintergreen oil), Kaligreen (potassium bicarbonate) and Nordox (cuprous oxide). Products were applied alone or in alternation with Nordox. Nu-Film-17, a non-ionic, terpene-based adjuvant was added to some products according to label instructions in 2008. The following fungicides products were tested in 2009: Kaligreen, Oxidate (hydrogen dioxide), Serenade Max, Sonata, Nu-Film-P by itself, JMS Stylet Oil, and different programs alternating these fungicides. Nufilm P, an organic formulation was used as an adjuvant with most products. In both years, a conventional standard fungicide program and the fungicide Pristine (pyraclostrobin and boscalid: not organic) were added to evaluate disease control compared to what would have been possible with conventional fungicides.

Treatments were applied to 3-vine plots and were replicated four times in a randomized complete block design. Sprays were applied with an R&D Research CO_2 cart-styled sprayer equipped with six bottles (3 L each) and a single XR TeeJet 8002VS nozzle on a 1.5-m spray boom until 17 Jun. After this date, applications were made using a research sprayer with high- flow electric pump with t-jet nozzles. Spray volume was 373 L/ha through 17 Jun then 467 L/h for the remainder of the season. Spray dates and approximate phenological stages in 2008 were as follows: 17 May (2-5 cm shoot), 29 May (7-12 cm shoot), 17 Jun (25-40 cm shoot), 30 Jun (1st post-bloom), 17 Jul 2007 (2nd post-bloom, bunch closure), 30 Jul 2007 (3rd post-bloom), 11 Aug (4th post-bloom). In 2009, spray dates and approximate phenological stages were as follows: 18 May (2-8 cm shoot); 27 May (15-20 cm shoot); 23 Jun (1st post-bloom); 7 Jul (2nd post-bloom, bunch closure); 21 Jul 2007 (3rd post-bloom); 11 Aug (4th post-bloom); 18 Aug (5th post bloom).

On Aug 4, 25 randomly chosen leaves per plot were visually assessed for downy mildew incidence (% leaves with disease symptoms) and severity (% area affected on diseased leaves only). Overall severity was calculated as (incidence x severity)/100. Vines were also monitored for signs of phytotoxicity. In 2009, disease incidence and severity were evaluated on 30 leaves per plot for downy mildew on 9 Sep and for powdery mildew on 24 Sep. Data were analyzed by ANOVA followed by mean separation using Fisher's Protected LSD (alpha is 0.05) in the StatGraphics program. Data were transformed as needed.

Downy mildew pressure was high in 2008 due to heavy rains in June and early July. The disease also showed up relatively early in the growing season and led to severe defoliation by the time of harvest. No powdery mildew was seen in 2008. In 2008, the conventional standard program showed the best performance against downy mildew but Sonata and Serenade were statistically similar (data not shown). Serenade alternated with Nordox, Sonata alternated with Nordox, and Neu 1160 Vegetable Oil alternated with Nordox, and Pristine (not organic) also provided good control. Other treatments were less effective, although all of them were better than the untreated control. The biofungicides Serenade and Sonata were the most promising for control of downy mildew. Both fungicides were generally equivalent to each other. NuFilm adjuvant may play a role in disease control as well, as this adjuvant by itself has also shown disease control capability. In 2009, downy and powdery mildew were moderately severe. Downy mildew was controlled best by the standard program, followed by Pristine, Serenade alternated with Sonata, Serenade and Sonata (Table 1). Other treatments tended to be less effective, but still were a significant improvement over the untreated control. All treatments, significantly reduced overall severity of powdery mildew (Table 2) including the adjuvant Nu-Film-P. The best results were obtained with the conventional fungicide program, but a program alternating four different biocontrol agents also provided very good control. In addition, Serenade, Sonata, and Serenade alternated with Sonata performed well.

Based on the above results, we feel that organic growers have viable options for control of downy and powdery mildew in grapes. Serenade and Sonata are the most promising products overall; however, the addition of Nu-Film-P may be important to improve efficacy. There did not appear to be a substantial difference between the full and half rates of Serenade and Sonata, which might make these product more economical but still effective when used at a reduced rate. A regular, preventative spray schedule and thorough coverage are critical for optimizing control for these fungicides, since all of these products are contact materials.

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Table 1: Efficacy of organic fungicides against downy mildew in grapes (cv. Niagara) in Fennville, MI, USA in 2009.

Treatment, product rate/hectare	Application timing[z]	Downy mildew on leaves							
		Incidence (%)		Severity (%)		Overall severity [%]		Control [%][w]	
Untreated		91.3	a[y]	50.4[x]	a	46.7[x]	a		
Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	71.3	bc	29.0	bc	22.8	bc	[51.2]	
Kaligreen 5.6 kg + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	70.0	bcd	24.1	bcd	17.2	bcd	[63.2]	
Oxidate 1:100 v/v + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	63.8	bcde	20.0	bcde	13.3	cde	[71.5]	
JMS Stylet Oil 9.33 L	1, 2, 3, 4, 5, 6, 7	50.0	efg	19.0	cde	10.2	defg	[78.2]	
Sonata 9.33 L+ Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	58.8	bcdefg	15.3	de	9.7	defg	[79.2]	
Serenade Max 3.4 kg + Nu-Film-P 0.58 L Kaligreen 5.6 kg JMS Stylet Oil 9.33 L Sonata 9.3 L + Nufilm P 0.58 L	1, 2, 3, 4, 5, 6, 7	57.5	cdefg	16.2	de	9.6	defg	[79.4]	
Serenade Max 1.7 kg + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	51.3	defg	16.1	de	8.8	defg	[81.1]	
Sonata 4.67 L + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	52.5	cdefg	15.0	de	8.4	efgh	[82.0]	
Serenade Max 3.4 kg + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	52.5	cdefg	15.3	de	8.1	efgh	[82.7]	
Serenade Max 3.4 kg + Nu-Film-P 0.58 L Sonata 9.33 L + Nu-Film-P 0.58 L	1, 3, 5, 7 2, 4, 6	47.5	efg	18.1	cde	8.0	efgh	[82.9]	
Pristine 38WDG 0.56 kg (**not organic**)	1, 2, 3, 4, 5, 6, 7	42.5	g	12.2	e	5.4	fgh	[88.4]	
Dithane Rainshield 3.4 kg Abound 2.08F 0.88 L Nova 40WP 0.35 kg +Ziram 3.4 kg Phostrol 3.5 L (**not organic**)	1, 2, 3, 4, 5, 6, 7	22.5	h	11.4	e	2.4	h	[94.9]	

[z]Spray dates: 1 = 18 May (2-8 cm shoot); 2 = 27 May (15-20 cm shoot); 3 = 23 Jun (1st post-bloom); 4 = 7 Jul (2nd post-bloom, bunch closure); 5 = 21 Jul 2007 (3rd post-bloom); 6 = 11 Aug (4th post-bloom); 7 = 18 Aug (5th post bloom).

[y]Column means followed by the same letter are not significantly different according to Fisher's Protected test ($P \leq 0.05$).

[x]Values shown are actual means; statistical analysis was performed on square root(x)-transformed data.

[w]Bracketed values denote percent control relative to the untreated check.

Table 2: Efficacy of organic fungicides against powdery mildew in grapes (cv. Niagara) in Fennville, MI, USA in 2009.

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Treatment, Product rate/hectare	Application timing[z]	Powdery mildew on leaves							
		Incidence (%)		Severity (%)		Overall severity [%]		Control [%][w]
Untreated		89.0	a[y]	31.1[x]	a	28.1	a	
Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	80.7	a	20.7	bc	17.5	b	[37.7]
JMS Stylet Oil 9.33 L	1, 2, 3, 4, 5, 6, 7	64.2	b	14.8	cde	10.4	c	[63.0]
Oxidate 1:100 v/v + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	60.0	bc	14.3	cd	8.9	c	[68.3]
Kaligreen 5.6 kg + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	47.8	cde	12.1	def	5.9	cd	[79.0]
Serenade Max 1.7 kg + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	52.8	bcd	10.0	defg	5.3	cd	[81.1]
Serenade Max 3.4 kg + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	36.3	efg	7.4	fghi	3.1	d	[89.0]
Sonata 9.33 L + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	38.8	def	8.0	efgh	2.9	d	[89.7]
Serenade Max 3.4 kg + Nufilm P 0.58 L Kaligreen 5.6 kg JMS Stylet Oil 9.33 L Sonata 9.33 L + Nufilm P 0.58 L	1, 5, 7 2, 3, 4, 6,	33.0	fgh	6.6	ghi	2.2	d	[92.2]
Sonata 4.67 L + Nu-Film-P 0.58 L	1, 2, 3, 4, 5, 6, 7	31.2	fgh	6.3	ghi	2.1	d	[92.5]
Serenade Max 3.4 kg + Nu-Film-P 0.58 L Sonata 9.33 L + Nu-Film-P 0.58 L	1, 3, 5, 7 2, 4, 6	28.4	fghi	4.6	hi	1.4	d	[95.0]
Pristine 38WDG 0.56 kg (**not organic**)	1, 2, 3, 4, 5, 6, 7	31.2	fgh	3.9	hi	1.4	d	[95.0]
Serenade Max 3.4 kg + Nu-Film-P 0.58 L Kaligreen 5.6 kg + Nu-Film-P 0.58 L	1, 5, 7, 9 4, 6, 8,	31.0	fgh	3.2	hi	1.0	d	[96.4]
Dithane Rainshield 3.4 kg Abound 2.08F 0.88 L Nova 40WP 0.35 kg + Ziram 3.4 kg Phostrol 3.5 L (**not organic**)	1, 2, 3, 4, 5, 6, 7	14.8	i	4.5	hi	0.5	d	[98.2]

[z]Spray dates: 1 = 18 May (2-8 cm shoot); 2 = 27 May (15-20 cm shoot); 3 = 23 Jun (1st post-bloom); 4 = 7 Jul (2nd post-bloom, bunch closure); 5 = 21 Jul 2007 (3rd post-bloom); 6 = 11 Aug (4th post-bloom); 7 = 18 Aug (5th post bloom).

[y]Column means followed by the same letter are not significantly different according to Fisher's Protected test ($P \leq 0.05$).

[x]Values shown are actual means; statistical analysis was performed on square root(x)-transformed data.

[w]Bracketed values denote percent control relative to the untreated check.

List of Authors

List of Participants

1. Ahmed Sophia
sofia.ahmed@bordeaux.inra.fr
INRA- Bordeaux
Villenave d'Ornon, France

2. Al Turaihi Emad
al_turaihi@yahoo.com
Ministry of Environnement
Doha, Qatar

3. Angeli Dario
dario.angeli@iasma.it
IASMA, Research and Innovation Centre, FEM
San Michele all'Adige, Italy

4. Anthony Somers
anthony.somers@industry.nsw.gov.au
Industry and Investment NSW
Paterson, Australia

5. Armstrong Hugh
hugh.armstrong@bayercropscience.com
Bayer CropScience
Basket Range SA, Australia

6. Armstrong Jodie
jodie@invis.com.au
Integrated Viticultural Solutions
McLaren Flat. SA. Australia

7. Audeguin Laurent
laurent.audeguin@vignevin.com
IFV
Le Grau du Roi, France

8. Aumont Christophe
christophe.aumont@basf.com
BASF
Ecully, France

9. Austin Craig
cna8@cornell.edu
Cornell University
Geneva, NY, USA

10. Aveline Nicolas
nicolas.aveline@vignevin.com
IFV
Blanquefort, France

11. Barthe Muriel
muriel.barthe@vins-bordeaux.f
CIVB
Bordeaux, France

12. Berkett Lorraine
lorraine.berkett@uvm.edu
University of Vermont
Burlington, VT, USA

13. Bernard Farnçois
bernar@vignevin.com
IFV
Blanquefort, France

14. Blasi Paule
paule.blasi@colmar.inra.fr
INRA- Colmar
Colmar, France

15. Bleyer Gottfried
gottfried.bleyer@wbi.bwl.de
Staatliches Weinbauinstitut
Freiburg, Germany

16. Boso Alonso Susana
susanab@mbg.cesga.es
Mision Biologica de Galicia
Pontevedra, Spain

17. Braybrook David
dbraybrook@swin.edu.au
Research & Development Solutions
Wonga park, Australia

18. Breth Karl
weingut.breth@t-online.de
Weingut Breth
Alsheim, Germany

19. Caffi Tito
tito.caffi@unicatt.it
Università Cattolica S. Cuore
Piacenza, Italy

20. Calonnec Agnès
calonnec@bordeaux.inra.fr
INRA- Bordeaux
Villenave d'Ornon, France

21. Carisse Odile
carisseo@agr.gc.ca
Agriculture and AgriFood Canada
St-Jean-sur-Richelieu, Canada

22. Cartolaro Philippe
cartolaro@bordeaux.inra.fr
INRA- Bordeaux
Villenave d'Ornon, France

23. Claverie Marion
marion.claverie@vignevin.com
IFV
Orange, France

24. Collina Marina
mcollina@agrsci.unibo.it
University of Bologna
Bologna, Italy

25. Corio-Costet Marie-france
coriocos@bordeaux.inra.fr
INRA- Bordeaux
Villenave d'Ornon, France

26. Dagostin Silvia
silvia.dagostin@iasma.it
Fondazione Edmund Mach-IASMA
San Michele all'Adige, Italy

27. Davy Alexandre
alexandre.davy@vignevin.com
IFV
Blanquefort, France

28. Delière Laurent
laurent.deliere@bordeaux.inra.fr
INRA-Bordeaux
Villenave d'Ornon, France

29. Delmotte François
delmotte@bordeaux.inra.fr
INRA- Bordeaux
Villenave d'Ornon, France

30. Diez Ana
adiez@neiker.net
Neiker-Tecnalia
Arkaute, Spain

31. Dubuis Pierre-Henri
pierre-henri.dubuis@acw.admin.ch
Agroscope Changins-Wädesnwil
Nyon, Switzerland

32. Dufour Marie-Cécile
dufour@bordeaux.inra.fr
INRA
Villenave d'ornon, France

33. Dula Terézia
terezia@dulabor.hu
Bayer Hungária Kft.
Budapest, Hungary

34. Elia Natacha
n.elia@gironde.chambagri.fr
Chambre d'Agriculture de la Gironde
Blanquefort,France

35. Emmett Robert
Bob.Emmett@dpi.vic.gov.au
Department of Primary Industries
Mildura, Australia

36. Evans Katherine
Katherine.Evans@utas.edu.au
University of Tasmania
New Town, Australia

37. Gadoury David
dmg4@ cornell.edu
Cornell University, New York State Agricultural Experiment Station
Geneva, NY, USA

38. Gamm Magdalena
magdalena.gamm@dijon.inra.fr
University of Bourgogne
Dijon, France

39. Gessler Cesare
cesare.gessler@agrl.ethz.ch
ETH
Zurich, Switzerland

40. Giosue Simona
s.giosue@horta-srl.com
Horta Srl, Spin off company of Università Cattolica S. Cuore
Piacenza, Italy

41. Girard Magdalena
magdalena.girard@charente-maritime.chambagri.fr
Chambre d'Agriculture
La Rochelle, France

42. Giraud Frédéric
fgiraud@staphyt.fr
Biorizon/Staphyt
Martillac, France

43. Godfrey Dale
dale.godfrey@adelaide.edu.au
The University of Adelaide
Glen Osmond, Australia

44. Gubler Doug
wdgubler@ucdavis.edu
Cornell University
New York, USA

45. Gutard Sylvain
sylvain.guittard@vignevin.com
IFV
Blanquefort, France

46. Heloir Marie-Claire
mcheloir@dijon.inra.fr
University of Bourgogne
Dijon, France

47. Huerga Vanesa
vhuerga@neiker.net
Neiker-Tecnalia
Arkaute, Spain

48. Hufnagl Andrea
AHufnagl@dow.com
Dow AgroSciences
Mougins, France

49. Jaworska Grazyna
Grazyna.Jaworska@fra.dupont.com
Du Pont de Nemours SAS
Nambsheim, France

50. Jelev Zvezdomir
zvezdoss@yahoo.com
Agricultural University
Plovdiv, Bulgaria

51. Jermini Mauro
mauro.jermini@acw.admin.ch
Agroscope Changins-Wädenswil ACW
Contone, Switzerland

52. Jiang Lu
jiang.lu@famu.edu
Center for Viticulture and Small Fruit Research
Tallahassee, FL, USA

53. Jianhua Liu
ljh0779@sina.com
Institute of Plant and Environment Protection
Beijing, China

54. Jiye Yan
jiyeyan@gmail.com
Institute of Environment and Plant Protection
Beijing, China

55. Kassemeyer Hanns-Heinz
hanns-heinz.kassemeyer@wbi.bwl.de
Staatliches Weinbauinstitut
Freiburg, Germany

56. Kast Walter K.
wkkast@aol.com
State Research Institut for Viticulture, Oenology and Fruit Technology
Weinsberg, Germany

57. Kortekamp Andreas
andreas.kortekamp@dlr.rlp.de
Dienstleistungszentrum Ländlicher Raum Rheinpfalz
Neustadt, Germany

58. Labourdette Gilbert
gilbert.labourdette@bayercropscience.com
Bayer CropScience
Lyon, France

59. Lafond David
david.lafond@vignevin.com
IFV
Beaucouzé, France

60. Lagouarde Patrice
Patrice.lagouarde@bayercropscience.com
Bayer CropScience
Lyon, France

61. Latinovic Nedeljko
nlatin@ac.me
Biotechnical Faculty
Podgorica, Montenegro

62. Latorse Marie-Pascale
marie-pascale.latorse@bayercropscience.com
Bayer CropScience
Lyon, France

63. Legler Sara Elisabetta
saraelisabetta.legler@unicatt.it
Università Cattolica S. Cuore
Piacenza, Italy

64. Leroy Pascal
leroy@ivry.inra.fr
INRA-Ivry
Ivry-sur-Seine, France

65. Machefer Virginie
virginie.machefer@bordeaux.inra.fr
INRA- Bordeaux
Bordeaux, France

66. Magarey Peter
pmagarey@riverland.net.au
Magarey Plant Pathology
Loxton, Australia

67. Martinez Rodriguez Carmen
carmenmartinez@mbg.cesga.es
Mision Biologica de Galicia
Pontevedra, Spain

68. Merdinoglu Didier
didier.merdinoglu@colmar.inra.fr
INRA- Colmar
Colmar, France

69. Mestre Pere
pere.mestre@colmar.inra.fr
INRA- Colmar
Colmar, France

70. Miclot Anne-Sophie
anne-sophie.miclot@colmar.inra.fr
INRA- Colmar
Colmar, France

71. Mliki Ahmed
ahmed.mliki@cbbc.rnrt.tn
Centre de Biotechnologie de Borj-Cédria
Hammam-Lif, Tunisie

72. Moyer Michelle
mmm78@cornell.edu
Cornell University, New York State Agricultural Experiment Station
Geneva, NY, USA

73. Naud Olivier
olivier.naud@cemagref.fr
Cemagref
Montpellier, France

74. Palmieri Cristina
mariacristina.palmieri@iasma.it
IASMA, Research and Innovation Centre, FEM
San Michele all'Adige, Italy

75. Perazzolli Michele
michele.perazzolli@iasma.it
IASMA, Research and Innovation Centre, FEM
San Michele all'Adige, Italy

76. Pertot Ilaria
ilaria.pertot@iasma.it
IASMA, Research and Innovation Centre, FEM
San Michele all'Adige, Italy

77. Polverari Annalisa
annalisa.polverari@univr.it
University Verona
Verona, Italy

78. Raynal Marc
marc.raynal@vignevin.com
IFV
Blanquefort, France

79. Reuveni Moshe
mreuveni@research.haifa.ac.il
Golan Research Institute, University of Haifa
Katzrin, Israel

80. Reynaud Catherine
creynaud@domainelatapy.com
Domaine Expérimental La Tapy
Carpentras-Serres, France

81. Rossi Vittorio
vittorio.rossi@unicatt.it
Università Cattolica S. Cuore
Piacenza, Italy

82. Schilder Annemiek
schilder@msu.edu
Michigan State University
East Lansing, MI, USA

83. Schnee Sylvain
sylvain.schnee@bordeaux.inra.fr
INRA- Bordeaux
Bordeaux, France

84. Schröder Stephan
stephan.schroeder@kit.edu
Karlsruhe Institute of Technology
Karlsruhe, Germany

85. Scott Eileen
eileen.scott@adelaide.edu.au
University of Adelaide
Adelaide, Australia

86. Seem Robert
rcs4@cornell.edu
Cornell University
Geneva, NY, USA

87. Serrano Eric
eric.serrano@vignevin.com
IFV
Lisle sur Tarn, France

88. Smits Nathalie
smits@supagro.inra.fr
INRA-Montpellier
Montpellier, France

89. Steiger Dominique
dominique.steiger@bayercropscience.com
Bayer CropScience SAS
Monheim am Rhein, Germany

90. Tremblay Mathieu
Mathieu.Tremblay@agr.gc.ca
Agriculture and AgriFood Canada
St-Jean-sur-Richelieu, Canada

91. Veres Anikó
veres.aniko@mkk.szie.hu
Szent István University, Institute of Genetics and Biotechnology
Gödöllö, Hungary

92. Verpy Antoine
gdoncfd@yahoo.fr
Gdon du Libournais
Saint-Emilion, France

93. Wicks Trevor
trevor.wicks@sa.gov.au
Sardi
Adelaide, Australia

94. Wiedemann- Merdinoglu Sabine
sabine.merdinoglu@colmar.inra.fr
INRA
Colmar, France

95. Xinghong Li
lxh1962@yahoo.com.cn
Institute of Environment and Plant Protection
Beijing, China

96. Zah-Bi Iritché Cyrille
zahbi@evry.inra.fr
INRA-CNRS-University of Evry
Paris, France

97. Zahavi Tirtza
tirtzaz@yahoo.com
Ministry of Agriculture
Ramat Hagolan, Israel

98. Zanzotto Allessandro
alessandro.zanzotto@entecra.it
C.R.A.Istituto Sperimentale per la Viticoltura
Conegliano, Italy

Meeting organization

Agnès Calonnec, INRA Bordeaux-Aquitaine
François Delmotte, INRA Bordeaux-Aquitaine
Marc Raynal, IFV Bordeaux-Aquitaine

Local organization

Jean-Marc Armand
Philippe Cartolaro
Laurent Delière
Marie Lauwerier
Marie-Christine Médalin
Pierre Sauris and
Alain Girard

Address

INRA Bordeaux-Aquitaine
Umr Santé Végétale (INRA-ENITA)
Institut des Sciences de la Vigne et du Vin
71, avenue Edouard Bourlaux
B.P. 81
33883 Villenave d'Ornon cedex
France

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