The main challenges of viticulture : global warming, biotic and abiotic stress

Serge Delrot

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COST 858 Viticulture
Final Meeting

Bordeaux
October 27-30, 2009

What's up in viticulture ?

SESSIONS :

Grape genomics and its use in the field

Control of grape maturity and systems biology

Effects of environment and viticultural practices on grape berry quality

Grapevine diseases

Vineyard stress, field practices and genetic resources

Delegates from :

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Cyprus
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Hungary
Israel
Italy
Luxemburg
New-Zealand
Poland
Portugal
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Spain
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Switzerland
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Venue : Agora du Haut Carré
rue du Haut Carré
33400 Talence, France

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COST 858 Viticulture
Final Meeting

Bordeaux
October 27-30, 2009

What's up in viticulture ?

Book of abstracts

Coordinateur

Pr Serge DELROT

UMR « Ecophysiology and Functional Genomics »

Institute of Sciences for Vines and Wines

210, chemin de Leysotte, F-33883 Villenave d'Ornon, France

Foreword

This abstract book compiles the contributions presented during the Final Meeting of COST Action 858 «Viticulture - biotic and abiotic stress - grapevine defence mechanisms and grape development » held in Bordeaux, October 27-30, 2009.

In addition to a major financial support by COST, this meeting also received funding from the Conseil Régional d'Aquitaine, the Conseil Interprofessionnel du Vin de Bordeaux (CIVB) and INRA, which are constant supporters of our lab and of the Institut des Sciences de la Vigne et du Vin Bordeaux Aquitaine (ISVV). The Institut Fédératif de Recherches 103, which gathers all the laboratories involved in plant research on the INRA campus of Bordeaux also provided additional funding. I wish to thank them all very sincerely for this support.

Many colleagues in my lab (UMR 1287 Ecophysiology and Grape Functional Genomics) and in ISVV also facilitated the venue of this meeting, by taking in charge many organisational aspects. I cannot list all of them, because it would be too long, but special thanks are due to Dr. Nathalie Ollat who invested a lot of time, enthusiasm and energy to handle many organisational and scientific aspects, including the preparation of this abstract book, to Catherine Chabirand and Claude Bonnet for handling the budget, meals, transportation and secretarial aspects, to Pr. Kees van Leeuwen for finding some good examples of our favorite research topic, and for organizing the vineyard trip together with Elisa Marguerit.

The idea of the Action, which was submitted to COST and accepted in 2002 originated from discussions I had with Dr. Michel Boulay and with Jean-Pierre Mégnin (Moet Company). COST 858, which actually started at the end of 2003, aimed to create an active network of scientists working in different scientific areas (ecophysiology, agronomy, plant physiology, cellular and molecular biology, phytopathology, genetics, chemistry) that may contribute to help the competitiveness of European viticulture. According to OIV, grapevine is the most cultivated fruit tree worldwide, and Europe is ranked number 1 all over the world for viticultural area (4,139,975 ha = 55%), grapes (29,050,923 t = 43%) and wine (191,015,000 hl = 67%) production. Wine also represents a multicultural heritage which has grown since several milleniums in Europe and later spread to other continents. Viticulture and oenology are a subtle blend of science and art, tradition and innovation. There is a huge diversity in grapevine genomes (rootstocks and scions), climates, soils, viticultural practices and wine-making processes. This results in a tremendous range of wine types all around the world.

However, in spite of its historical, cultural and economical value, viticulture must face several challenges. Global climate change already impacted significantly grapevine physiology and berry composition in the last decades. Should the present climatic trend continue in the next decades, we may have to switch to varieties that are better adapted to new climatic conditions, which may affect the traditional typicity characterizing given regions.

National and European regulations tend to be more and more restrictive regarding the use of phytochemical treatments, and the consumer is paying more and more attention to environmentally safe practices. In this context, new methods aiming at a strong reduction of phytochemical treatments (but maintaining yield and typicity) must be designed. They include new viticultural practices, precision viticulture, sophisticated alert systems based on epidemiological studies, use of compounds that may stimulate natural plant defence, biological pest control, and new grapevine genotypes obtained by breeding.

Several scientific and technical achievements occurred during the duration of COST 858, that facilitated its initial goals. The sequence of the grapevine genome, released in 2007, was a major breakthrough that boosted grapevine research. It facilitated gene mapping, allowed the design of performing microarrays, and the adaptation of tools mapping the transcriptional data on metabolic pathways. Continuous improvements in high throughput DNA and RNA sequencing, gene expression studies, metabolomic analysis now result in the development of systems biology approaches, which require a major investment in bioinformatic tools. This will undoubtly make it easier to face the practical challenges of adaptation to climate change and pesticide limitation, provided that these new tools are constantly combined with field observations and measurements. All these aspects will be illustrated during the meeting.

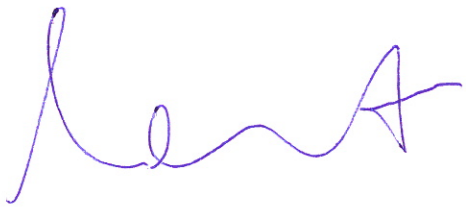
Like for many other things in life, it is never easy to say whether you failed, succeeded, or to what extent you succeeded. We were initially 9 participating countries, and we are now 17 European countries, and Australia and New Zealand also joined the Action. USA started this year a Grape

Research Coordination Network (GRCN) funded by NSF, roughly on the same basis (function and aims) as COST 858. Several delegates of GRCN attend the present meeting. All this may indicate that COST 858 was successful, but there are still major challenges to face: (a) transfer this enormous amount of scientific progress to the vineyard, to the grape growers and to the wine industry (b) to deal with societal issues related to wine consumption. The European paradox is that worldwide wine consumption increases, whereas European consumption decreases. Wine is an alcoholic beverage, and should obviously be drunk in moderate amounts. However, wine tasting has nothing to do with binge drinking, and it may have potential beneficial effects for health, in addition to being a cultural vehicle. These issues may be the topic of another COST action.

COST 858 allowed me to develop new interactions and ideas, to make many new contacts, new friends, and I hope it was the same for many participants. I am intimately convinced that this type of initiative really paves the way to a true and sincere European spirit, by catalyzing meetings and exchanges between different cultures, different scientific areas, different generations. This is only possible by the strong commitment and dedication of the COST officers who really try to help as much as they can. Dr. Wolfgang Obert, and then Dr. Albino Maggio, as Scientific Officers, and Jeannette Nchung Oru, as Administrative Officer were in charge of COST 858 in Brussels. They deserve very warm thanks and regards, for always answering our numerous administrative and scientific requests, and providing us adequate funding. Last but not least, this is also only possible because of you, COST participants, who devoted part of your time to the Action, especially the Vice-Chairs (Pr. Luigi Bavaresco and Kalliopi Roubelakis-Angelakis), the Work Group leaders, the colleagues who organized meetings, workshops, training schools throughout Europe (2 or 3 every year), members of the Management Committee, and of the Short Term Scientific Mission committee, senior scientists and STSM participants.

In addition to this abstract book, a book entitled "Methods and results in grapevine research" issued from COST 858 activities, will be published next year, with the financial support of COST.

Pr. Serge Delrot
COST 858, Chair



COST 858 Viticulture
Final meeting, Bordeaux, 27-30 october 2009

What's up in viticulture ?

Location: Agora du Haut Carré, rue du Haut Carré, 33400 Talence, France

Scope
The final meeting of COST 858(Viticulture) will stress the activities of the network during the past 6 years and show how it can help to solve current problems in viticulture: adaptation to environment, grapevine diseases. We will take the opportunity to interact with the Research Coordinated Network on Grapevine that was recently set up in the USA for a 5-year period.

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Acknowledgments

We would like to warmly thank the donators for their kind gifts of wines for this event.
The wines which we will be tasting during this conference are:

Château Beychevelle 2001
St Julien, 4ème Grand Cru Classé
Kindly offered by the property

Chateau Bonnet 2008
Entre Deux Mers,
kindly offered by Conseil Interprofessionnel des Vins de Bordeaux

Château Clos Fourtet 2002
St Emilion Premier Grand Cru Classé B
Kindly offered by the Conseil des Vins de St Emilion

Château la Conseillante 2001
Pomerol
Kindly offered by the property

Domaine de Couhins 2006
Pessac Léognan – Cru Classé
Kindly offered by Institut National de la Recherche Agronomique

Cave de Bédagan Cuvée "Le Grand Art"
Médoc
Kindly offered by the Cave Coopérative de Bédagan

Château Farluret 2007
Barsac
Kindly offered by Conseil Interprofessionnel des Vins de Bordeaux

Château Fonroque 2004
St Emilion Grand Cru Classé
Kindly offered by the Conseil des Vins de St Emilion

Château la Garde 2004
Pessac Léognan
Kindly offered by Conseil Interprofessionnel des Vins de Bordeaux

Domaine du Grand Parc 2005
Premières Côtes de Bordeaux
Kindly offered by Institut National de la Recherche Agronomique

Château Guiraud 2002
Sauternes Premier Grand Cru Classé
Kindly offered by the property

Château Haut Vallade 2004
St Emilion Grand Cru
Kindly offered by the Conseil des Vins de St Emilion

Château Lagrange 2004
St Julien, 3ème Grand Cru Classé
Kindly offered by the property

Château Laville 2005
Sauternes
Kindly offered by the property

Château Pontet Fumet 2005
St Emilion Grand Cru
Kindly offered by the property

Château Puisseguin Curat 2004
Puisseguin
Kindly offered by the Conseil des Vins de St Emilion

Château Reclos de la Couronne 2004
Montagne St Emilion
Kindly offered by the property

Château Val d'Or 2004
St Emilion Grand Cru
Kindly offered by the Conseil des Vins de St Emilion

Château d'Yquem 1998
Sauternes, Premier Grand Cru Classé Supérieur
Kindly offered by the property

For the floral decoration

Le GIE Plantes et Fleurs du Sud Ouest

Contents

Program

Tuesday, October 27, 2009

8h30- 9h45 *Registration + welcome coffee*

9h45-10h00 Serge Delrot: Welcome and introduction: the main challenges of viticulture: global warming, biotic and abiotic stress

10h00-12h05 Grape genomics and its use in the field.

Chair: Eva Zyprian

10h00-10h20: Riccardo Velasco (Italy): From genome sequence to MAS in viticulture: no more a dream?

10h20-10h40: Sean Myles (USA): Sequencing and SNP genotyping in the USDA grape germplasm collection

10h40-11h00: Erica Mica (Italy): An integrated approach for the characterization of miRNAs in grapevine

11h00-11h20: Massimo Delledonne (Italy): Whole genome microarrays and mRNA-Seq for expression analysis in *Vitis vinifera*

11h20-11h35: Patrice This (France): Study of grape allelic diversity at large scale for the analysis of complex traits in grape

11h35-11h50: Kristina Gruden (Slovenia): Extending Mapman ontology for grapevine

11h50-12h05: Etti Or (Israël): Recent advances in the understanding of the regulation of bud dormancy release

12h05 – 14h00 *Lunch + poster viewing*

14h00- 18h00 Control of grape maturity and systems biology

Session I. ***Chair: Charles Romieu***

14h00-14h25: Chris Davies (Australia): Understanding and manipulating the hormonal control of berry ripening

14h25-14h45: Claudio Moser (Italy): Regulation of berry ripening

14h45-15h05: Nancy Terrier (France): Flavonoid metabolism in grape berry

15h05-15h25: Luigi Bavaresco (Italy): Recent advances in stilbene metabolism of grapevine

15h25-15h45: Brian Jordan (New Zealand): Sauvignon Blanc grape berry biochemistry and molecular biology: the role of light, development and environmental stress

15h45-16h00: **Amnon Lichtner (Israel)**: Pre and postharvest management of table grapes

16h00-16h45 *Coffee Break and poster viewing*

Session II. ***Chair: Hipolito Medrano***

16h00-16h20: Grant Cramer (USA): Why systems biology is the way of the future?

16h20–16h40: Jérôme Grimplet (USA/Spain): VitisNet: "Omics" data integration through grapevine molecular networks

16h40-17h00: Mario Pezzotti (Italy): Berry ripening and withering system biology using integration of transcriptomic, proteomic and metabolomic data

17h00-17h20: Hugo Pena-Cortes (Chile/Germany): Integrated analysis of transcriptome and metabolome to understand grapevine development and its relation to wine attributes

17h 20-18h00: General discussion. From genomics and systems biology to the vineyard

18h00-20h00: *Welcome cocktail in Agora du Haut Carré*

Wednesday, October 28, 2009

8h30: *Transfer by bus from Agora Haut Carré to the Institute of Vine and Wine Sciences*

9h00- 12h00: Visit of the Institute of Vine and Wine Sciences

Welcome addresses
Visit of laboratories, genetic resources, experimental plots
Wine tasting

12h00- 13h30 *Lunch in the Institute of Vine and Wine Sciences*

13h30: *Transfer by bus to the Conference Center (Agora Haut Carré)*

14h00- 17h40 Effects of environment and viticultural practices on grapevine quality

Session I. ***Chair: Grant Cramer***

14h00-14h20: Georg Meisner (Germany): Evaluation of different viticultural farming systems with specific reference to biodynamic viticulture and the use of biodynamic preparations

14h20-14h40: Hans Schultz (Germany): Climate change and viticulture – challenges ahead

14h40-15h00: Jaume Flexas (Spain): Physiological responses of grapevine to water stress: implications for stress monitoring and effects on water use efficiency

15h00-15h20: Cornelis Van Leeuwen (France): C^{13} measurements to monitor the water status of grapevine.

15h20-15h40: Felicidad de Herralde (Spain): Rootstock influence on growth, water use efficiency and fruit quality

15h40-16h00: Nathalie Ollat (France): Rootstock/scion interactions: defining accurately the role of each partner

16h00-16h30: *Coffee break and poster viewing*

Session II. ***Chair: Chris Davies***

16h30-16h50: Kalliopi Roubelakis-Angelakis (Greece): What is new on the role(s) of Polyamines in the response of plants to stresses

16h50-17h10: Simone Castellarin (Italy): Impact of water deficit on flavonoid composition in Merlot grapes

17h10-17h25: Andrea Schubert (Italy): Transgenic grapevines overexpressing the aquaporin VvPIP2 ;4 show modified growth and water transport in irrigated and water stress conditions

17h25-17h40: Gyula Varadi (Hungary): The effect of vintage and terroir on thermal stability of grapevine leaf photosynthesis

18h00: *Departure by bus to Château Kirwan (Grand Cru Classé, Margaux) from Agora du Haut Carré*

Gala dinner

19h45: Visit of a cellar and wine tasting

20h45: Gala dinner

22h45: Return to downtown Bordeaux

Thursday, October 29

9h00-12h00 Grapevine diseases.

Session I. ***Chair: Riccardo Velasco***

9h00- 9h20: François Delmotte (France): When population genetics highlight the epidemiology of grapevine downy and powdery mildew

9h20-9h40: Lance Cadle-Davidson (USA): Effect of environment and genotype on disease sensitivity

9h40-9h50: Bertrand Léger (France): The GrapeMilDeWS Experiment: signposting one path to treat less and according to epidemics at the plot level

9h50-10h10: Vincent de Rudnicki (France): NTIC, new tools to control phytochemical treatments and traceability

10h10-10h45 *Coffee break and poster viewing*

Session II. ***Chair: Eric Gomes***

10h45 – 11h05: Didier Merdinoglu (France): Grapevine breeding for downy mildew resistance

11h05-11h25: Eva Zyprian (Germany): Diversity in oïdium resistance regions and differentially expressed genes

11h25-11h45: Jean-Marc Neuhaus (Switzerland): Beta-aminobutyric acid-induced resistance in grapevine

11h45-13h30 *Lunch and poster viewing*

13h30- 15h30: Vineyard stress, field practices and genetic resources.

Chair: Brian Jordan

13h30-13h50: Christian Chervin (France): Reduction of gray mold in grapes by ethanol applications

13h50-14h20: Sophie Bordiec (France): *Burkholderia phytofirmans* strain PsJN primed the expression of stress-related genes in *Vitis vinifera* L. upon low non-freezing temperatures

14H20-14h40: Carlos Lopes (Portugal): Cover cropping and deficit irrigation strategies: effects on water relations, growth and fruit composition in Tempranillo grapevines

14h40-14h55: Carlos Miranda (Spain): Water stress management in "Tempranillo" vineyards aiming at high quality. Interest of deficit irrigation strategies

14h55-15h10: Andreas Doulis (Greece): Identification of wine *Vitis vinifera* genotypes, autochtonous to Crete, Greece, employing ampelographic, AFLP and SSR markers

15h10-15h25: Claire Arnold (Switzerland): Genetic and chemical investigation of Swiss grape varieties

15h25-16h00: General Discussion and conclusions: New tracks and tricks to limit vineyard stress and maintain quality

For the members of the COST 858 Management committee only:

16h30-20h30: Management Committee Meeting

Summary of Work Group Progress 2003-2009
Preparation of the Final Report: Workshops, Training Schools, STSM, etc…

Friday October 30, 2009

8h30-17h00: Post-workshop vineyard trip in Saint-Emilion: traditional versus biodynamic viticulture, wine tasting

8h30: Departure by bus from Bordeaux
9h30: Visit of Chateau Cheval Blanc vineyard/cellar (1 group) or Chateau Figeac (1 group): traditional viticulture
11h00-12h00: visit of Saint-Emilion city

12h00-14h00: *Lunch and wine tasting in Saint-Emilion*

14h00 -15h30: Visit of Chateau Fonroque (1 group) and 1 château in Lussac (1 group), both run with "biodynamics" methods
17h00: arrival in Bordeaux

End of meeting

List of oral presentations

Effects of environment and viticultural practices on grapevine quality

Grapevine diseases

Vineyard stress, field practices and genetic resources

List of posters

ORAL PRESENTATIONS

THE MAIN CHALLENGES OF VITICULTURE: GLOBAL WARMING, BIOTIC AND ABIOTIC STRESS

S. Delrot,

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The present challenges that must be faced by European viticulture are shared with other continents. Viticulture and wine production must face an increasing competition and part of it must (may) adapt to potential new markets. However, in the same time European wines must maintain their typicity and quality in spite of the climatic change. Grapegrowers should also take into account the increasing concern of consumers and the more restrictive legal constraints on the use of phytochemicals.

For France, the Intergovernmental Panel on Climate Change forecasts a rise in mean temperature of +2.4°C, a decrease in summer rainfall, a 30% increase in potential evapotranspiration and a doubling of atmospheric CO_2 by the end of this century. Similar trends may be forecast for other European countries. This may obviously affect the physiology of grapevine, fruit yield and composition, sensitivity to diseases, and ultimately wine quality and typicity. Harvest occurs sooner and sooner, although grapegrowers tend to wait longer for ripeness. Berry sugar content (and alcohol in the wine) tend to increase whereas phenolic and aromatic ripeness are not always achieved. Acidity tends to decrease with potential effects on wine ageing capacity. Water supply is becoming shorter in many regions, and there is an increasing competition from many human activities for limited water resources. Global warming might even lead to change the usual and traditional varieties grown in some places, which will alter wine typicity. To face all these constraints, many possibilities may be explored, and some will be illustrated during this meeting. Viticulture practices including new methods of limited irrigation, vineyard exposure, vineyard density and leaf cover management are possible solutions. The systematic study of clonal diversity and design of new rootstocks provide other solutions which may be helped by the (re)sequencing of the grapevine genome(s) and by the use of molecular markers. Ultimately, changing the grapevine varieties presently grown may be necessary in some regions if the actual trend of climate change extends for a long time.

Another major challenge is to limit phytochemical treatments to meet the European regulations which are more and more restrictive. On one hand, global warming and extended drought may decrease the impact of some fungal diseases spreading under humid conditions, but on the other hand, it may also affect the natural resistance of the plants. Decrease of phytochemical consumption can be obtained by several viticultural practices, among improvement of alert systems and spraying devices are the most promising. Vigour is another parameter relevant for the susceptibility to some diseases. In the next years, genetic selection is also expected to deliver several varieties that would combine resistance to the three major diseases (*Erysiphe necator*, *Plasmopara viticola* and *Botrytis cinerea*). Alternative strategies aiming to elicit the general resistance of the plant give good results in controlled conditions (greenhouse), but their efficiency seems more erratic in the vineyard. Finally, biological fight may be envisaged to limit phytoplasma spreading insects or to limitt fungal attacks. « Pesticide free » viticulture and biodynamics are increasingly popular, but their economical sustainability is not yet demonstrated in the long range.

The availability of the grapevine genome, the fast improvement of hightroughput DNA sequencing and gene expression methods offer new opportunities to explore the genetic diversity of grapevine and to identify key genes controlling major physiological processes such as ripening or plant defense. However, to make full use of these progresses, to foster the emerging systems biology of grapevine, and to be able to better control these processes, there is a strong need for organizations collaborations related to genome annotation, joined data bases, bioinformatic tools and high throughput and highly sensitive analytical methods (metabolomic platforms) which may apply to grape berry content and wine composition.

FROM GENOME SEQUENCE TO MAS IN VITICULTURE: NO MORE A DREAM?

R. Velasco

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The Grapevine Genome Initiative, a collaboration between the Istituto Agrario di San Michele all'Adige – Fondazione Edmund Mach and two private companies, Myriad Genetics Inc. and the 454 Life Science, has focused on sequencing the elite cultivar Pinot Noir to provide unprecedented insight into the structural nature of heterozygosity in an outcrossing species. Of special interest to biologists and breeders are polymorphisms in and around the coding regions representing a substantial resource for molecular breeding programs, as well as trait and QTL marker association. In addition, this clone was a good model to study the transferability of its polymorphism content across individuals belonging to the same species and genus. The combining of genome shotgun of paired reads produced by Sanger sequencing and pyrosequencing of unpaired reads was shown to be an efficient procedure for decoding a complex genome. The coverage of SBS reads was crucial for identifying polymorphic sites. Over 2 millions of single nucleotide polymorphisms have been revealed by merging the two genomic haplotypes present in the Pinot noir genome. The availability of two haplotypes, joint with a further haplotype produced by the French-Italian consortium supplies the community of unprecedented tools for whole genome analysis toward association mapping and LD definition. Instruments like new generation sequencers, and high throughput genotyping systems like High Resolution Melting or iScan (Illumina), create opportunities to plan long term assisted breeding strategies.

GENETIC STRUCTURE OF THE GENUS VITIS

S. Myles[1*], J.-M. Chia[2] , B. Hurwitz[2], C. Simon[3,4], G. Y. Zhong[3,4], D. Ware[2,4], E. Buckler[1,4]

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Next-generation sequencing technologies promise to dramatically accelerate the use of genetic information for crop improvement. As genotyping costs decrease, the focus in genetic mapping studies is shifting from genotyping to experimental design. The first step in optimizing the design of genetic mapping studies involves large-scale polymorphism discovery and a subsequent genome-wide assessment of the population structure and pattern of linkage disequilibrium (LD) in the species of interest. From 2.6Gb of DNA sequence from 17 grape DNA samples (10 cultivated *Vitis vinifera* and 7 wild *Vitis* species) we identified 71,397 high-quality single nucleotide polymorphisms (SNPs). We chose 8988 of the discovered SNPs to be assayed by a custom Infinium genotyping array (the Vitis9KSNP array) and we have genotyped over 1800 grapevine accessions from the USDA grape germplasm repository with the Vitis9KSNP array. We demonstrate that *Vitis vinifera* has low LD even at short ranges. Together with the high levels of diversity observed in the grapevine, this observation suggests that well-powered genome-wide association studies in the grapevine will require whole-genome sequencing rather than microarray-based genotyping. We also provide a detailed view of the genetic relationships among *Vitis vinifera* cultivars and among species of the genus *Vitis* using phylogenetic trees, bayesian clustering analyses (STRUCTURE) and principal components analysis (PCA).

AN INTEGRATED ANALYSIS OF THE MIRNA GENES IN THE GRAPEVINE GENOME: STRUSCTURE, FUNCTION AND EXPRESSION PATTERN.

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MicroRNAs (miRNAs) are key post-transcriptional regulatory elements of eukaryotic gene expression. These small RNA molecules, approximately 21 nt long, are coded by MIRNA genes and transcribed by RNA polymerase II that gives rise to the miRNA-primary transcript (pri-miRNA) which is processed into the mature and active miRNA. This smallRNA plays a crucial role in many physiological and biological processes, such as plant development and defense against abiotic or biotic stresses, regulating the expression of transcription factors or key genes of methabolic pathways. Their critical role shows clearly that structural and functional characterization of any genome cannot exclude the analysis of miRNAs and small RNA population.

Here we present an approach towards the functional and structural characterization of miRNA genes in the grapevine (*Vitis vinifera* L.) genome, originated from the VIGNA (Vitis Genome Analysis) project that brought to the annotation of 140 conserved MIRNA genes (Jaillon et al., 2007). Starting from the description of the transcriptional landscape of these genes, obtained with both an RNA-seq and a microarray approach, we further analyzed their genomic structure and their interactions with putative target transcripts.

In particular we present an experimental validation of transcription boundaries and alternative splicing events of some MIRNA genes, previously predicted with a bioinformatic approach, that takes advantage from polyA$^+$ deep-sequencing data produced within the VIGNA consortium. This method confirmed bioinformatic predictions, and it shows different splicing patterns for different pri-miRNAs, and alternative transcription end points for the same primary transcript.

Moreover, we have validated some candidate target genes using the modified 5'Rapid Amplification of cDNA Ends (RACE) protocol, to detect *in vivo* RNA cleavage products. In particular we have focused on target genes that seems to be up regulated in root tissues during microarray experiments, involved in secondary metabolites synthesis processes. The same target genes could be potentially involved in berry maturation processes and further analyses and validation is in progress, to assess their function in this agronomically important trait. Upcoming results will augment our understanding of miRNA gene structure and function in the grapevine genome.

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WHOLE GENOME MICROARRAYS AND MRNA-SEQ FOR EXPRESSION ANALYSIS IN *VITIS VINIFERA*

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The Italy-France Grape Genome Consortium is ready to release the 12X assembly of the *Vitis vinifera* genome. For functional genomics studies, the international community now needs a whole transcriptome grape microarray. We have developed two microarrays based on the Gene Prediction V1 produced by the Italy-France Grape Genome Consortium. The two microarrays, which are based CombiMatrix and Nimblegen technologies, monitor the expression of about 29,500 genes out of the 29,971 annotated genes. I'll present the result of the validation process for the two microarray platforms, as well as my personal view of the advantages/disadvantages of analysing gene expression by Combimatrix, Nimblegen or RNA-seq.

We have conducted the first global analysis of grapevine (*Vitis vinifera* cv. Corvina) transcriptome during berry development by RNA-seq, and setup a pipeline that works for biologists, not just for bioinformaticians. The cDNAs libraries obtained by retrotrascription of fragmented mRNA are sequenced with an Illumina Genome Analyzer IIx. By using two different softwares for alignment of sequences on the reference genomes (ELAND and BOWTIE) the short reads (36 or 75 nucleotides, single reads or paired ends) are mapped to exons, introns or intergenic regions, hence identifying new exons or new genes not predicted. We have adapted modules of the ERANGE program for digital gene expression analysis, detection of alternative splicing (annotated or new) and SNPs discovery. Expression values, calculated as reads per kilobase of exon model per million mapped reads (RPKM) are then used to monitor changes in gene expression. We are currently evaluating several statistical approaches to identify genes which are significantly modulated.

STUDY OF GRAPE ALLELIC DIVERSITY AT LARGE SCALE FOR THE ANALYSIS OF COMPLEX TRAITS IN GRAPE

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The genomic revolution have been the starting point for the renewal of attention on the extend of natural occurring variation in many plants species and in particular in the model species *Arabidopsis thaliana* (Alonso-Blanco and Koornneef 2000). Because grape is experimenting similar genomic breakthroughs, the use of genetic diversity in this species may greatly improve our understanding of quantitative economically important traits. The sequencing of the grape genome (Jaillon et al, 2007; Velasco et al, 2007) indeed opened the door for more extensive analysis of grape allelic diversity.

In the past decade, thanks to an important international effort, our understanding of grape nuclear diversity greatly improved (This et al. submitted), in particular through European Programs such as GenRes81 and GrapeGen06 (http://www1.montpellier.inra.fr/grapegen06/accueil.php) leading to the standardization of SSR analysis (This et al 2004). Our knowledge of grape diversity in genes, is however still limited. Even if the number of SNPs revealed on ENTAV 115 Pinot genome is very high (Velasco et al. 2007). The SNP diversity varies between 1 SNP per 129 bases up to 1 SNP per 49 bases (Lijavetzky et al. 2007, Salmaso et al. 2004, Le Cunff et al. 2008) but until now he estimation has been analyzed either on small number of cultivars or small numbers of genes. .

In the present work, analysis of gene diversity over a sample of 47 accessions of *Vitis vinifera*, and several species of *Vitis* have been estimated for a set of 800 genes scattered through the grape genome. Through bioinformatics developments, diversity map of the grape genome has been drawn. This data will be very useful in order to select markers for mapping and fror comparison of diversity between *Vitis* species.

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EXTENDING MAPMAN ONTOLOGY FOR GRAPEVINE

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Whole genome transcriptomics analysis is a very powerful approach because it gives an overview of the activity of genes in certain cells or tissue types. However, biological interpretation of such results can be rather tedious. MapMan is a software tool that displays large datasets (e.g. gene expression data) onto diagrams of metabolic pathways or other processes and thus enables easier interpretation of results. The grapevine (*Vitis vinifera*) genome sequence has recently become available bringing a new dimension into associated research. Two microarray platforms were designed based on the TIGR Gene Index database and used in several physiological studies.

To enable easy and effective visualization of those and further experiments, annotation of *Vitis vinifera* Gene Index (VvGI version 5) to MapMan ontology was set up. Due to specificities of grape physiology, we have created new pictorial representations focusing on three selected pathways: carotenoid pathway, terpenoid pathway and phenylpropanoid pathway, the products of these pathways being important for wine aroma, flavour and colour, as well as plant defence against pathogens. This new tool was validated on Affymetrix microarrays data obtained during berry ripening and it allowed the discovery of new aspects in process regulation. We here also present results on transcriptional profiling of grape plantlets after exposal to the fungal pathogen *Eutypa lata* using Operon microarrays including visualization of results with MapMan. The data show that the genes induced in infected plants, encode pathogenesis related proteins and enzymes of the flavonoid metabolism, which are well known as being responsive to fungal infection.

The extension of MapMan ontology to grapevine together with the newly constructed pictorial representations for carotenoid, terpenoid and phenylpropanoid metabolism provide an alternative approach to the analysis of grapevine gene expression experiments performed with Affymetrix or Operon microarrays. MapMan was first validated on an already published dataset and later used to obtain an overview of transcriptional changes in a susceptible grapevine – *Eutypa lata* interaction at the time of symptoms development, where we showed that the responsive genes belong to families known to be involved in the plant defence towards fungal infection (PR-proteins, enzymes of the phenylpropanoid pathway).

RECENT ADVANCES IN THE UNDERSTANDING OF THE REGULATION OF BUD DORMANCY RELEASE

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Hydrogen cyanamide (HC) provides controlled, synchronized and relatively rapid induction of grape bud dormancy release within a well-characterized time frame, thereby creating a traceable and reliable system that facilitate the identification of various biochemical components possibly involved in mechanism of grape bud dormancy release. Using this system, we previously demonstrated changes in the expression of various genes that suggested the development of oxidative and respiratory stress in the bud. A comparative approach was than adopted and showed that the same genes are similarly induced by another dormancy release stimulus, heat shock (HS), also in different timing and intensity. These findings, which suggest that similar mechanisms are triggered by different stimuli, led to a large-scale transcriptomic analysis of bud response to HC and HS in an attempt to expose the factors and pathways involved in dormancy release. Based on the outcome of this analysis, a surprising similarity is exposed of bud response to that of submerged plants. This similarity led to a new link in the field of bud dormancy release between sub lethal stress, perturbation of mitochondrial activity, development of hypoxic metabolism, ethylene:ABA interplay and cell enlargement. A new working model will be presented for the cascade that lead from the stimuli application to dormancy release, and post genomic analyses that support a central role for ethylene signaling in the regulation of dormancy release will be presented.

UNDERSTANDING AND MANIPULATING THE HORMONAL CONTROL OF BERRY RIPENING

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A central goal of our work is to better understand the process of grape berry ripening and how it is controlled in order to offer the grape and wine industries some new tools for management in the vineyard. Despite being of pivotal importance there is still much that is unknown about the control of berry ripening and the literature on this subject is somewhat confusing and in some cases contradictory (Davies and Böttcher, 2009). Recent microarray data has shown that the transition into ripening is accompanied by large changes in gene expression with the transcription of many genes being either up or down regulated. It is assumed that the coordination of these events occurs, in the main part, as a result of hormone signalling. In this paper an update on our current thinking regarding the hormonal control of berry ripening will be given. Two aspects will be considered, i.e. which hormones are likely to be involved in the 'normal' process of ripening and which hormones may be useful for manipulating ripening. This will include a discussion of the abilities of some hormones, e.g. abscisic acid, to hasten ripening (Wheeler *et al.*, 2009) and others, e.g. auxins, to delay ripening. In particular, recent work on the role of indole-3-acetic acid (IAA)-amido synthetases will be outlined. These enzymes appear to act during grape development to sequester free IAA and may play a crucial role in reducing IAA levels, thereby enabling berry ripening.

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REGULATION OF BERRY RIPENING

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Grape berry ripening is a complex process which involves the activation of many different metabolic pathways encompassing among the others, sugars and acids metabolism, secondary metabolism and cell wall metabolism. The outcomes of these processes determine fruit quality and therefore the final market value of grapes and grape-derived products (mainly wine). Beside equally important scientific reasons, understanding grape berry ripening regulation is of great economic importance and it has been subject of investigation for the last forty years. Auxin, brassinosteroids, abscic acid and ethylene, likely in concert rather than alone, have been reported to be the hormones mainly affecting berry ripening. According to classical physiology,the grape berry is considered as a non-climacteric fruit, but a growing number of evidences suggest that ethylene in fact may play a role in this process.

To understand the importance of ethylene signalling during berry development and ripening, we measured ethylene concentration in Pinot Noir grapes and we identified the grape putative homologues of the Arabidopsis and tomato genes known to be involved in ethylene synthesis, perception and signalling. The expression profiles of ACC synthase (ACS), ACC oxidase (ACO), ethylene receptors (ETRs) and ethylene response factors (ERFs) genes clearly showed isogene-specific differences depending on tissue and developmental stage. Further functional characterization of selected candidates is in progress and it will help to understand the role of ethylene in the onset of grape berry ripening.

The occurrence of an oxidative burst at the onset of fruit ripening has been reported for several plants, both climacteric and non. We also observed a strong accumulation of hydrogen peroxide (H_2O_2) in Pinot Noir grapes around vèraison. Recent works propose a signalling role for reactive oxygen species (ROS) in developmental processes such as seed and bud dormancy release and floral transition. In the attempt to better characterize the oxidative burst observed in grapes, we measured H_2O_2 accumulation and lipid oxidation state in berry skin and pulp, separately and we observed significant differences. Finally, ROS scavenging capacity has been characterized at the biochemical level in order to explain the transient nature of the oxidative stress.

FLAVONOID METABOLISM IN GRAPE BERRY

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Flavonoids are a large group of secondary metabolites involved in plant defense and reproduction. *Vitis vinifera* appears as a particularly interesting model to study the biosynthesis of flavonoids.

Within the EU project FLAVO, different approaches of metabolomics, transcriptomics, plant transformation and genetics have been coupled in order to decipher the missing steps in the flavonoid pathway. A transcription factor controlling the PA biosynthetic pathway in the seed, called MybPA1, had already been identified in grapevine (Bogs *et al*., 2007). We have identified a new Myb factor called VvMybPA2. VvMybPA2 presents strong homologies with other plant Myb factors regulating the flavonoid pathway and is expressed mainly in the skin of green berries. Transgenic hairy roots of *Vitis vinifera* overexpressing either VvMybPA1 or VvMybPA2 under the 35S promoter exhibited qualitative and quantitative modifications of their proanthocyanidin contents. *Vitis* transformants overexpressing transcription factors inducing flavonoid synthesis were constructed and analyzed at the phenotypic and transcriptomic levels, allowing the identification of new putative actors of the anthocyanin and PA pathways (Cutanda-Perez *et al*., 2009; Terrier *et al*., 2009). Among the genes that were associated with anthocyanin accumulation, we identified a gene presenting a strong homology with a putative vacuolar anthocyanin transporter, and named it anthoMATE3 (*AM3*). *In vitro* studies demonstrated that AM3 protein acts *in vitro* as vacuolar H^+-dependent acylated anthocyanin transporters, suggesting that anthoMATE play a role in the vacuolar anthocyanin transport in grapevine (Gomez *et al*., 2009). A collection of *Vitis* cultivars and a segregating population were screened for their flavonoid composition. Several QTLs were detected and interestingly one region is involved in the control of the mean degree of polymerization of skin proanthocyanidin.

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RECENT ADVANCES IN STILBENE MATABOLISM OF GRAPEVINE

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Stilbenes are low molecular weight phenolics with antifungal activity, thus enabling plants to cope with pathogen attack. Grapevine stilbenes include a number of substances, such as resveratrol, resveratrol glucosides (piceid and resveratroloside), viniferins, pterostilbene, piceatannol, astringin, pallidol, and other resveratrol trimers and tetramers.

These compounds are induced (phytoalexins) in non-woody plant organs/tissues (leaves, flowers, berries and other non-woody organs) whereas they are constitutively synthesized in woody and hard (seeds) ones. The most studied and best known stilbene is resveratrol. It was first detected in 1940 as a root constituent in the white hellebore lily (*Veratrum grandiflorum* O. Loes) although the richest source is *Polygonum cuspidatum*, an Asian medicinal plant.

Resveratrol is also present in wine and is claimed to be involved in the positive health effects arising from wine drinking, including the reduction of risk from heart disease, from cancer and from other diseases.

When grapevine is under biotic and/or abiotic stress, stilbenes act as phytoalexins. Some of these stresses are natural, occurring at different extents in commercial vineyards, others are artificially applied in order to improve the stilbene content (Bavaresco *et al.*, 2009).

All viticultural factors are involved in regulating stilbene synthesis, that is the grape variety, the climate, the soil, the cultural practices.

Current scientific research on stilbenes is aiming at investigating the factors involved in the gene (stilbene synthase) expression (Gatto *et al*., 2008) and in finding agronomical methods to improve resveratrol concentration in the plant, especially in the grapes. It is of further interest to study the role of stilbenes in the plant metabolism.

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SAUVIGNON BLANC GRAPE BERRY BIOCHEMISTRY AND MOLECULAR BIOLOGY: THE ROLE OF LIGHT, DEVELOPMENT AND ENVIRONMENTAL STRESS

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New Zealand has 40-50% higher levels of UV-radiation compared to similar regions in the northern hemisphere. The unique environment of high light intensity, clear skies and high UV can have a profound influence on the biochemical composition of plants. Little is known, however, about the effects of UV on the biochemistry of New Zealand grape vine cultivars and on the resulting wines. The relationship between leaf and berry biochemistry is also poorly understood, particularly in the context of the light environment. An important aspect of this research programme is to improve the understanding of the LOX-HPL pathway as it plays a critical role in stress responses and the biosynthesis of important aroma compounds. During the last two seasons UV screens have been placed over rows of Sauvignon Blanc grapes in the Lincoln Vineyard to study the effects of UV-B and UV-A radiation in 'leaf plucked' vines and in comparison to control vines (no screens and no leaf removal). UV-exposure clearly had a dramatic effect on the physical appearance of the berries with specific pigmentation relating to the extent of exposure. Pigmentation does, however, also have a temporal component as it develops post veraison. Substantial biochemical changes took place on UV exposure, particularly in both qualitative and quantitative flavonoid composition. The levels of certain amino acids in the berries relate to the presence of leaves remaining over the fruiting zone. This is very significant because amino acids are important components in wine making as the precursors for phenolics, thiols, methoxypyrazines and yeast available nitrogen (YAN). Wines have been made from these treatments and show differences in appearance and taste from the initial pressings to the final product. We are also characterising the LOX-HPL biochemical pathway. At least four LOX genes were found to be expressed in berries. Real-time PCR analysis of the berry expressed LOXs revealed differential tissue distribution and a different expression pattern during development. Individual LOX genes showed strong induction upon wounding and in response to pathogen infection. We have cloned one of the berry-expressed LOXs, *VvLOXA*, into a protein expression vector and expressed it as a recombinant fusion with His-tag in *E. coli*. The recombinant VvLOXA protein was partially purified and assayed with a range of substrates. We have determined basic kinetic characteristics and optimum assay conditions of the recombinant protein *in vitro*. The HPLC analysis of the VvLOXA products suggested the enzyme is a 13-LOX. We are currently continuing the biochemical and molecular characterisation of other members of the LOX gene family.The observed changes in biochemical composition are of significance for wine making and need further understanding of the regulatory mechanisms involved.

PRE AND POSTHARVEST MANAGEMENT OF TABLE GRAPES IN A HOT CLIMATE

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Growing table grapes in a hot climate presents developmental challenges and significant consequences to fruit quality after harvest. Growing table grapes under shade nets is one way to reduce radiation: it is shown that this practice can alleviate sun damage and reduce fruit decay after harvest. High temperature during berry maturation prevents color development of red cultivars: it is shown how application of abscisic acid enables growing of table grapes in regions not optimal for cultivation of colored varieties. In addition, it is shown that an *ex-situ* assay can facilitate the study of color development in the laboratory. Hot climate can also have consequences on infection of table grapes by toxicogenic fungi: it is described how these fungi persist through cold storage. Finally, hot climate can have a positive effect on fruit infection by *Botrytis cinerea*: the implications of this fact on the postharvest treatments of table grapes are described. In summary, growing of table grapes under hot climate conditions requires the use of pre and postharvest means which do not necessarily apply to table grape viticulture in temperate climate.

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WHY SYSTEMS BIOLOGY IS THE WAY OF THE FUTURE ?

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A plant is a complex organism made up of many organelles, cells, tissues and organs, all of which work in harmony with each other. There are more than 250,000 plant species displaying a wide diversity of traits. Complex traits are influenced by many small quantitative trait loci (QTLs) indicating complex interactions with a lot of factors. Plant phenotypes are explained by genotype and environment interactions. With so many genes, proteins, metabolites and environmental variables the possible interactions are nearly infinite.

Modern plant biology is rapidly changing in the "post-genomic" era. The sequencing of the Arabidopsis genome has led to a bountiful array of information and molecular tools to improve our understanding of the expression of genes, proteins, and metabolites. Understanding the function of genes is a major challenge of the post-genomic era. Integrative functional genomics, also known as "Systems Biology", is an emerging discipline that takes the molecular parts (transcripts, proteins and metabolites) of an organism and attempts to fit them into functional networks or models designed to describe the dynamic activities of that organism.

To understand complex traits, one requires a large number of genotypes of a population in order to apply association mapping and systems biology approaches to help elucidate plant physiology and phenotype. The theory is in place, the tools are well developed and the data sets are emerging. It won't be long before these efforts will result in significant progress in plant breeding and crop production. Some relevant examples in biology will be presented.

VITISNET: "OMICS" INTEGRATION THROUGH GRAPEVINE MOLECULAR NETWORKS

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Genomic data release for the grapevine has increased exponentially in the last five years. The *Vitis vinifera* genome has been sequenced and *Vitis* EST, transcriptomic, proteomic and metabolomic tools and data sets continue to be developed. The next critical challenge is to provide biological meaning to this tremendous amount of data by annotating genes and integrating them within their biological context. We have developed and validated a system of Grapevine Molecular Networks (VitisNet). The sequences from the genome sequencing project and ESTs from the *Vitis* genus have been paired and the 39,424 resulting unique sequences have been manually annotated. Among these, 13,145 genes have been assigned to 219 networks. The pathway sets include 88 "Metabolic", 15 "Genetic Information Processing", 12 "Environmental Information Processing", 3 "Cellular Processes", 21 "Transport" and 80 "Transcription Factors". The quantitative data is loaded onto molecular networks, allowing the simultaneous visualization of changes in the transcriptome, proteome and metabolome for a given experiment. VitisNet uses manually annotated networks in SBML or XML format, enabling the integration of large datasets, streamlining biological functional processing, and improving the understanding of dynamic processes in systems biology experiments. VitisNet is grounded in the *Vitis vinifera* genome (currently 8x coverage) and can be readily updated with subsequent updates of the genome or biochemical discoveries.

BERRY RIPENING AND WITHERING SYSTEMS BIOLOGY USING INTEGRATION OF TRANSCRIPTOMIC, PROTEOMIC AND METABOLOMIC DATA

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Berries for sweet dessert wines (e.g. Recioto, Vin Santo) and dry fortified wines (e.g. Amarone) undergo a phase of post-harvest dehydration which can last up to three months, where metabolism is modified significantly and the sugar content increases. The molecular processes that occur during withering are poorly understood. The identification of the driving molecular events characterizing the ripening and withering of the *Vitis vinifera* cv. Corvina grapes is matter of interest for the production of Amarone and Reciotto wines.
A genome-wide transcriptional analysis, performed using a 24.471-gene chip, identified 12.285 transcripts expressed during 7 sampling time-points, covering the period from the pre-veràison until the completion of withering. The same sampling time-points were analyzed at proteomic and metabolic levels. Seven hundred and fifty-eight protein spots and four hundred and eight metabolites were identified by DIGE analysis and untargeted large-scale metabolomics (LC-MS) respectively.
We first performed an unsupervised PCA analysis on each dataset revealed that the 7 sampling time-points are grouped in 3 main classes (pre-veràison and veràison in the first class, pre-ripening and ripening in the second class and the three withering sampling time-points in the third one). On the basis of the PCA results, the class-specific variables were identified by O2PLS-DA analysis of each dataset. Transcript, protein and metabolite biomarkers for each class were then identified using a two-class O2PLS-DA analysis.
We finally integrated transcriptome, proteome and metabolome datasets by a series of O2PLS-DA analyses.

INTEGRATED ANALYSIS OF TRANSCRIPTOME AND METABOLOME TO UNDERSTAND GRAPEVINE DEVELOPMENT AND ITS RELATION TO WINE ATTRIBUTES

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Different physiological and biochemical processes are involved in fruit setting, development and ripening of grapevine berries. Diverse efforts using varied technologies are being applied in order to explain certain key biological stages of this fruit which could allow, in a near future, to improve the quality of grapevine berries and consequently the quality of wine.

To gain information concerning the genes and metabolites involved in such processes, we measured primary and secondary metabolites together with transcript levels of three *Vitis vinifera* cultivar berries during their growth period. Samples of cultivars Carmenère, Merlot and Cabernet Sauvignon were collected every three days and during two growth seasons, starting with flowers and finishing with mature berries. We established a qRT-PCR platform to investigate the transcriptional changes during growth development and setting that allows us to determine the expression levels of around 800 grapevine genes. We measured metabolite levels by using GC-TOF-MS and UPLC/FT-MS for primary and secondary metabolites respectively. Since huge amount of data is generated, we use a network analysis approach based on correlations as a tool to investigate the relationship between transcripts and metabolite changes and their role in regulation of metabolic pathways during ripening.

Understanding the processes involved in grape ripening will contribute to obtain better fruits quality which would help to improve wine quality. Considering the advantages provided by using LC-MS (to analyse secondary metabolites), we are applying this technology to investigate whether a non-targeted wine chemical composition analysis could provide suitable information on the relationship between the quality scores -given to Chilean commercial red wines by experienced winemakers- and wine matabolome data and in that way provide an objective method to discriminate wine quality scores and other wine attributes like variety, origin or vintage. The analysis of the metabolic profiles of the wine samples by unsupervised multivariate techniques and *in house* developed bioinformatics tools recognize signatures"(Biomarkers") in wine metabolome which allow to differentiate attributes like variety, vintage, origin and wine quality scores.

The knowledge acquired will allow to identify potential biomarker of quality present in the furit and the wine and will also allow to determine the correlation among them. Such kind of information can be used to improve agricultural techniques in order to obtain better quality fruits for better wines.

EVALUATION INTO DIFFERENT VITICULTURAL FARMING SYSTEMS WITH SPECIFIC REFERENCE TO BIODYNAMIC VITICULTURE AND THE USE OF BIODYNAMIC PREPARATIONS

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World wide many wine farms are converting into organic or biodynamic viticulture. The main reasons are better wine quality and healthier wine with the long-term aim of sustainability in viticulture.
In 2005 a long-term study started in Geisenheim. The objective of the research program is to investigate, compare and optimise the techniques of integrated, organic and particularly biodynamic wine production in terms of resource protection and food quality.
The goals of this study are to look at the effects on the biological and microbial activity in the soil, the vegetative and generative growth of the vine, microbiology, the grape and wine quality and the sustainability of the three viticultural systems.

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CLIMATE CHANGE AND VITICULTURE – CHALLENGES AHEAD

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The rapidly increasing world population and the scarcity of suitable land for agricultural food production together with a changing climate will ultimately put pressure on grape producing areas for the use of land and the input of resources. For most grape producing areas the predicted developments in climate will be identical to becoming more marginal for quality production and/or to be forced to improve resource management. This will have a pronounced impact on grapevine physiology, biochemistry and ultimately production methods. Research in the entire area of stress physiology, from the gene to the whole plant and vineyard level (including soils) will need to be expanded to aid in the mitigation of arising problems. Two major challenges can be identified, one related to the lack of knowledge about how plants will respond to a rise in CO_2 concentration, temperature and a possible lack of water simultaneously under field conditions, and the second on the broader aspect of resource management in the production chain of wine within the industry and possibilities for its improvement.

For the first challenge, the primary limitation is the establishment of sufficiently large infrastructures to simulate future climate developments such as increased CO_2 concentration and temperature under field conditions. In a recent editorial for the New Phytologist titled "an inconvenient truth" with reference to the Academy award for the best documentary film by former US Vice President Al Gore, Woodward (2007) described and analysed the dilemma between practical experiments with elevated CO_2 concentrations and the need to understand and predict the future responses of plants in the field. Aside from the fact that increasing CO_2 concentrations will impact on global temperature, CO_2 itself is generally beneficial to plant growth, although the response strongly varies between species (Long et al. 2004). However, Woodward (2007) continued that CO_2 enrichment experiments usually don't mimic the gradual increase in CO_2 plants are experiencing in the field, but rather follow a step-up approach, and possible differences in plant responses to these approaches are unknown. Additionally, CO_2 enrichment is not usually accompanied by warming as would be predicted by climate models because of "the problem of securing long-term funding which is a bothersome limitation to a more general approach" (Woodward 2007). Recent results from models including the physiological impact of CO_2 on plants (more biomass, reduced g) suggest that rising CO_2 will increase the temperature driven water evaporation from the oceans resulting in an increased absolute water vapour content of the air. However, the decrease in evapotranspiration over land (due to a decrease in stomatal conductance) would still lead to an overall decrease in relative humidity and to an increased evaporative demand according to current knowledge (Boucher et al. 2009). Plant surfaces should then heat up more due to stomatal closure adding to the complexity of expected responses difficult to trace and simulate in conventional experiments.

It is exactly this complexity which necessitates a more global approach to setting-up experimental systems to study the response of grapevines to the combined increase in temperature and CO_2, one of the biggest challenges ahead to understand. Few studies have investigated the response of grapevines to CO_2 either in small FACE (free air carbon dioxide enrichment) systems (Bindi et al. 1995, Bindi et al. 2001a) or in open top chambers (Gonçalves et al. 2009), but these could only describe the impact of increasing CO_2 concentration in the absence of rising air temperature. Nevertheless, the generally predicted increase in biomass was confirmed, yet the effects on water consumption remained unclear (Bindi et al. 1995, Bindi et al. 2001a). These experiments also showed that fruit sugar concentration should increase and acidity levels decrease under elevated CO_2 (Bindi et al. 2001b), but the response of other components contributing to flavour and aroma of grapes were heterogeneous and indicated a significant "chamber effect", with plants grown outside responding differently than plants in open top chambers with or without elevated CO_2 (Gonçalves et al. 2009).

The second challenge for the wine industry is more related to the management of natural resources in the production chain for wine and the resulting carbon or water footprints. Whereas the carbon footprint for entire regions has been roughly estimated (examples for the Champagne and Bordeaux regions, (CIVC, 2007; CIVB, 2009) and some strategies devised to reduce it, the water footprint is an upcoming issue which will affect agriculture in general. Water management is no longer an issue restricted to individual countries or river basins. Even a continental approach is not sufficient. The water footprint of Europe – the total volume of water used for producing all commodities

consumed by European citizens – has been significantly externalised to other parts of the world. Europe is for example a large consumer of sugar and cotton, two of the most thirsty crops (Hoekstra and Chapagain 2008). Rising food demand and growing water scarcity (IPCC 2008) will put increasing pressure on agriculture, which is currently using up about 70% of the world's fresh water resources for irrigation. Currently, issues such as the amount of water imported by a country through products (including the *direct* input of water used for its production and the *indirect* water used for services around this product (transport or packaging) are emerging in the context of water neutral production budgets of countries or sustainability strategies of super market chains. Spain, for instance, is exporting 189 Mm^3 water per year to the UK alone captured in products related to grape production (Chapagain and Orr 2008). Although these calculations and budgets have not yet had impacts on production strategies in the wine industry, the firsts signs are appearing in California and Australia and will ultimately have a feed-back effect on research related to irrigation management and water use efficiency strategies in viticulture. Additionally, the water issue can not be seen strictly independent from other climate related problems, since the release of nitric oxide and CO_2 from agricultural land contributes significantly to the "greenhouse effect", and since this release depends on soil water content, irrigation management and organic matter content (Avrahami and Bohannan 2009). For grape production, however, we have currently no information on the contribution and/or possible management strategies of these effects, another significant challenge for future research.

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PHYSIOLOGICAL RESPONSES OF GRAPEVINE TO WATER STRESS: IMPLICATIONS FOR STRESS MONITORING AND EFFECTS ON WATER USE EFFICIENCY

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In many viticultural areas, drought (i.e., soil water stress) is the main environmental factor limiting photosynthesis and respiration and, consequently, grapevine growth and yield. The present review summarizes our work on photosynthesis and respiration under water stress and recovery after re-watering, highlighting the implications for stress monitoring and water use efficiency.

Diffusion limitations to photosynthesis under most water stress are predominant, involving decreased stomatal but also mesophyll conductance to CO_2, an important but often neglected process. A general failure of photochemistry and biochemistry, by contrast, can only occur when daily maximum stomatal conductance (g_s) drops below 0.05-0.10 mol H_2O m^{-2} s^{-1} (Flexas *et al*. 2002). At the leaf level, water use efficiency (WUE) is increased by drought during the diffusional phase of photosynthesis limitation, but it is decreased during the biochemical phase. As for respiration, this is much less affected than photosynthesis under water stress, which leads to an increased respiration to photosynthesis ratio, therefore decreasing yield (Medrano *et al.* 2003), and making improvements in WUE at the plant level less marked – if any – than at the leaf level (Flexas *et al.* 2009a). A sustained stomatal closure upon alleviation of water stress appears as the main cause for slow and incomplete photosynthetic recovery during re-watering which, however, leads to a sustained increase in leaf-level WUE (Flexas *et al.* 2009b).

A corollary is that improving WUE at the leaf level may require imposing cycles of moderate water stress (i.e., keeping plants at the diffusional phase) followed by recovery, while improving WUE at the whole plant level may be more difficult and involve decreases in plant respiration. The first goal appears as the most achievable in the short term, and may be aided by physiologically-based monitoring of plant water status for irrigation scheduling. Given the predominant role of diffusional limitations in driving grapevine yield and WUE under water stress, and instruments related to water flow through plants – such as sap flow, IR-thermography or dendrometry – are envisaged as the most powerful indicators for monitoring plant water status, although some parameters related to leaf biochemistry / photochemistry – such as chlorophyll fluorescence or leaf reflectance – have been also proposed (Cifre *et al.* 2005).

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$\delta^{13}C$ MEASUREMENTS TO MONITOR THE WATER STATUS OF GRAPEVINE

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Vine water status has a major impact on grape composition and wine sensory attributes. Many techniques have been developed over the years to assess vine water status. Most of them need extensive field measurements, which limits their practical use for vineyard management. Carbon isotope discrimination ($^{13}C/^{12}C$ ratio, or $\delta^{13}C$) measured on grape sugar at harvest time is an integrative indicator of vine water status. It can be measured by mass spectrometry on grape juice and it does not need any intervention in the field other than grape sampling at harvest. Hence, many measurements can be carried out at a reasonable cost, making this method available for vineyard management purposes. In irrigated vineyards, high quality fruit is produced with fine tuned deficit irrigation. $\delta^{13}C$ measurements on grape sugar allow validating irrigation strategies by showing which plots received too little or too much irrigation water. $\delta^{13}C$ measurement provides wineries that purchase grapes with a practical tool to assess if deficit irrigation was implemented in the vineyard. Grape quality for red wine production is closely related to the level of water deficit the vines have been subject to during the growing cycle. $\delta^{13}C$ could be used as an indexation tool for fixing the price of the grapes. In dry farmed vineyards, $\delta^{13}C$ values are closely related to the water holding capacity of the soil, canopy architecture and the climate of the vintage. Vine water status is a major component of terroir expression. Because many measurements can be carried out at a reasonable cost, $\delta^{13}C$ is a powerful tool to map vine water uptake conditions and can thus be used in terroir zoning studies. Vine water deficit promotes shoot growth slackening, reduces berry size and increase grape anthocyanin content. Hence, these parameters are well correlated with grape $\delta^{13}C$ measured at ripeness.

ROOTSTOCK INFLUENCE ON GROWTH, WATER USE EFFICIENCY AND FRUIT QUALITY

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There are two main challenges for the viticulture in the 21st century. On one hand, there is the arising importance of biotechnology disciplines and systems biology that can provide new tools for the fundamental knowledge of the metabolic pathways and its integration together with physiology (Hayes, 2008, Roubelakis-Angelakis, 2009). On the other hand, the Global Change questions the continuity of many agronomical practices and the suitability of the growth of specific varieties in their traditional optimal areas. Climate Change may increase the temperature locally or generally. Under the worst scenarios, temperature may increase up to 4ºC and rainfall may decrease 10-40%, mainly in summer under Mediterranean conditions. However, not only Climate Change but also new technologies, regulations on carbon footprint or fluctuating demands of the market, among others, may promote new needs in viticulture. Among the possible actions to face Global Change, rootstocks can provide many solutions. Rootstock selection for good grapevine productivity and quality depends on edaphoclimatic conditions of the vineyard, affinity for the variety, pests' resistance, and adaptation to conditions of drought, salinity, waterlogging... Rootstocks provide differences in vine performance due to hydraulic characteristics and root distribution that influence water and nutrients supply to the cultivar conferring vigor and affecting ripening date. Despite the importance of nutrient and water uptake and the need of rootstocks to resist pathogens and to allow growth in certain *terroirs*, only the 6% of grapevine scientific literature in the last year has been devoted to root systems (ISI Web of Knowledge). Our work with different rootstocks under a variety of conditions and different water availabilities has showed a direct relationship with growth (Alsina 2008; de Herralde et al 2008, 2009; Fortea et al. 2009; Savé et al 2009), with hydraulic properties and water use efficiency (Alsina 2008; de Herralde et al, 2006, 2008; Pou et al. 2008) and fruit quality (Alsina 2008; de Herralde et al 2008, 2009) which are presented.

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SCION / ROOTSTOCK INTERACTIONS: DEFINING ACCURATLY THE ROLE OF EACH PARTNER

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Viticulture is currently facing several changes, including environmental and economic ones. Adaptations of cultural practices to these changes must be made in a sustainable way in order to protect the environment, human working conditions and economical health of the property. Rootstocks represent one of the most effective long-term uses of a biological control mechanism for an agricultural pest. Moreover they participate to environmental condition adaptation and may contribute, to some extent, to the control of vine vigour and yield (Jones et al. 2009). The complex relationships between root and shoot systems of grafted grapevine have been widely studied particularly in relation to the effect of rootstock genotype on scion development, both in adult vines growing in a vineyard and in young potted vines. Unlike shoots, roots have received much more limited attention in grapevine scion/rootstock studies, essentially because of methodological difficulties associated with observing and measuring root architecture, biomass, and growth in situ (Swanepoel et al. 1989). Only one report related to the effect of scion genotype on the root development of different rootstocks has been published (Oslobeanu 1978). Our knowledge of the underlying physiological mechanisms determining biomass allocation between the shoots and the roots of grafted plants, where scion/rootstock interactions have to be considered, remains poor and somewhat fragmented (Jensen et al. 2003). Several hypotheses have been proposed based upon trophic exchanges (including carbon, minerals and water), growth regulators and RNA trafficking between the two partners of the association (Ollat et al. 2003).

Here we present several examples to illustrate scion / rootstock interactions. They are related to growth control and dry matter allocation within the plant under control and drought conditions. Several hypotheses of control mechanisms have been tested and the results will be reported.

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WHAT IS NEW ON THE ROLE(S) OF POLYAMINES IN THE RESPONSE OF PLANTS TO STRESSES ?

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Generation of reactive oxygen species (ROS) in plant cells is a nodal response to stresses. Polyamines (PAs), whose homeostasis is spatially and temporally regulated in plants, including the grapevine (Paschalidis and Roubelakis-Angelakis 2005a, 2005b), contribute to ROS homeostasis since they inhibit the NADPH-oxidase-mediated generation of ROS in grapevine *in vitro* models (Papadakis and Roubelakis-Angelakis 2005) and also their oxidation by Polyamine oxidase (PAO) results to the generation of hydrogen peroxide (H_2O_2). Transgenic tobacco plants overexpressing *Zea mays PAO (*S-*PAO)* contain lower soluble (S), soluble hydrolyzed (SH) and pellet hydrolyzed (PH) Spd and Spm and the opposite is true for plants carrying the *PAO* gene in antisense orientation (A-*PAO*). In both transgenics PA homeostasis is partially re-established *via* increased expression of PA biosynthetic genes and the increased H_2O_2 in S-*PAO* is scavenged by the significantly enhanced antioxidant activity. Further increase of ROS in S-*PAO* plants results to lower quantum yield of photosystem II, higher ion leakage, lipid peroxidation and induction of DNA fragmentation. These effects are mimicked by supply of exogenous H_2O_2 (Moschou et al. 2008a). Thus, although the higher levels of H_2O_2 generated by overexpression of *PAO* are successfully scavenged by the concomitant activation of the antioxidant machinery, further increase of ROS becomes detrimental to cellular functions and induces the programmed cell death (PCD) syndrome. As a result, S-*PAO* plants exhibit increased sensitivity to salt stress, whereas the opposite is true for the A-*PAO* plants. The mechanism of PA participation in the generation of H_2O_2 and the reaction to stress is the secretion of Spd into the apoplast where it is oxidized by PAO. The generated H_2O_2, depending on its titers, signals either protective mechanisms or initiate PCD, in a dose-responsive manner. In an effort to test whether the above physiological role(s) of PAs apply to other plant species, *Vitis vinifera* cv Sultanina cell suspension cultures were used. Following treatment with NaCl the same pattern of increase in both, the PA titers and the biosynthetic activities of ADC and ODC as well as the Spd secretion into the apoplast was found, as it was the case with tobacco. Finally, our recent results suggest that Spd levels in salt stressed and control plants affect respiratory activity *via* the modulation of ROS levels. Taking all together, PAs homeostasis seems to be an important salt stress tolerance trait. The recently characterized novel peroxisomal PAO responsible for a full back-conversion pathway in *Arabidopsis* remains to be identified in grapevine (Moschou et al. 2008c), as well as if overexpression of *PAO* could result to pathogen tolerance as it does in tobacco (Moschou et al. 2009).

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IMPACT OF WATER DEFICIT ON FLAVONOID COMPOSITION IN MERLOT GRAPES – A FOUR-YEAR STUDY

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Flavonoids in red grapes have a central role in the determination of wine quality. Anthocyanins are the major contributors to wine color, and proanthocyanidins are responsible for astringency and bitterness. Anthocyanins and proanthocyanidins are synthesized from the same flavonoid precursors, though the synthesis of proanthocyanins occurs at earlier stages of fruit development than anthocyanins. Flavonoid content and composition vary extensively in response to viticultural practices and are sensitive to a combination of environmental factors, unique to each vintage. Water deficit can modify vine growth and berry composition. In this study, a late season water deficit was imposed on field growth "Merlot" vines from the onset of ripening until harvest, over four seasons (2004, 2005, 2007 and 2008). Anthocyanin and proanthocyanidin content of berry skin were compared between irrigated and stressed vines.

Plant water status was monitored weekly by measuring the midday stem water potential, and maintained constantly between -0.2 and -0.6 MPa in control vines and between -0.6 and -1.4 MPa in water deficit vines. Berry weight and yield per vine were significantly reduced by water deficit in all four seasons, nevertheless technological parameters of fruit quality such as sugar and acid concentrations remained unaffected.

Anthocyanin content (mg per berry) and concentration (mg per berry fresh weight) were significantly increased by water deficit. Total anthocyanins per berry increased by 23 to 42% in water deficit grapes, across the years. When the data were expressed in terms of concentration, differences between treatments were even higher (plus 40 to 69% in water deficit plants), due to the additional effect of inhibition of berry growth in water deficit plants.

The pattern of proanthocyanidin accumulation was similar under the two water regimes, with a peak of accumulation immediately before or at veraison. Proanthocyanidin content in the skin was not affected by water shortage in all seasons except 2004, when water deficit plants had significantly more proanthocyanidins at harvest. By contrast, concentration of skin proanthocyanidin, expressed relative to the whole berry weight (mg per berry fresh weight), was significantly higher in water deficit grapes in 2004, 2005 and 2008.

According to this study, anthocyanin biosynthesis is strongly promoted by water deficits imposed during the last stages of fruit development and throughout ripening. This gain was not achieved at the expense of the other class of flavonoids synthesised from the same precursors, the proanthocyanidins. In fact, proanthocyanidin synthesis was not competitively affected by the overexpression of anthocyanin biosynthetic genes in water stress plants, because the two processes are temporally sequential during fruit development and ripening (Castellarin et al 2007a and b). The increase in proanthocyanidin concentration observed in water stressed grapes was determined by the inhibition of berry growth and the lower berry weight.

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TRANSGENIC GRAPEVINES OVEREXPRESSING THE AQUAPORIN VVPIP2;4 SHOW MODIFIED GROWTH AND WATER TRANSPORT IN IRRIGATED AND STRESS CONDITIONS.

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We transformed grapevines to overexpress VvPIP2;4, a PIP-family aquaporin gene which enhances water transport across the plasma membrane in Xenopus oocytes. Several transformed lines with different levels of transgene expression were obtained. We tested wild-type and transformed lines under conditions of normal irrigation and under stress by measuring: leaf area, leaf gas exchanges, root hydraulic conductivity using a High Pressure Flow Meter (HPFM) apparatus, and leaf proline content. Furthermore we measured embolism formation and recovery in leaf petioles by determination of hydraulic conductivity before and after application of a transient flushing pressure.

In irrigated conditions, net photosynthesis, stomatal conductance, transpiration and leaf area were significantly higher in transgenic plants than in wild type plants. Higher stomatal conductance of transgenic plants was related to higher root hydraulic conductivity. Transgenic plants suffered petiole vessel embolization upon mild environmental conditions not causing embolism in wild-type plants, probably due to their transpiration levels higher than in wild type plants. Proline content was significantly higher in transgenics than in wild type plants.. Under water stress, water transport parameters were less affected by expression of the transgene, and in these conditions transformation did not induce significant modifications of growth and embolization intensity.

THE EFFECT OF VINTAGE AND TERROIR ON THERMAL STABILITY OF GRAPEVINE LEAF PHOTOSYNTHESIS

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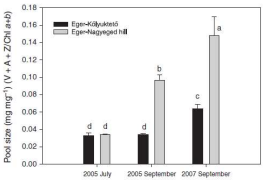
Heat sensitivity of grapevine (*Vitis vinifera* L. cv. Kékfrankos) photosynthesis was studied in two vineyards (Eger-Kőlyuktető, flat; and Eger-Nagyeged hill, steep slope) with different mesoclimates and water supply conditions in two climatically different years. 2007 was drier and warmer, with higher vapour pressure deficit (VPD) than 2005. Pre-dawn water potential measurements indicated mild water deficit at the steep-sloped vineyard. In July 2005 mild water deficit enhanced the thermostability of grapevine photosynthesis, as reflected in the temperature dependence of optimal quantum yield (F_v/F_m) and in the critical temperature of initial fluorescence (F_0T_c). Decreased F_v/F_m and actual quantum yield ($\Delta F/F_m'$) was recorded at most temperatures in September at the water-stressed (steep slope) site. This time, F_0T_cs were also lower due to early leaf senescence. In September 2007, heat sensitivity of F_v/F_m was similar to 2005, and $\Delta F/F_m'$ indicated higher thermostability at both sites, but keeping the consistent difference between the two vineyards. The critical points of steady-state fluorescence (F_sT_c) were higher by 3–6°C at both vineyards in 2007 than in 2005. Although, in September thermolabile F_0 signals were measured at the water-stressed vineyard, the heat sensitivity was not decreased in light adapted state, assumingly as a result of enhanced xanthophyll cycle pigment pool size. The long-term water deficit had a significant effect not only on yield and wine composition (Zsófi et al. 2009), but also on leaf pigment concentration (see Fig.). The higher xanthophyll pigments pool size (V + A + Z) in 2007 even at the mild stressed vineyard (black bars) suggests that high temperature and VPD play a role in increasing (V + A + Z)/(chl *a* + *b*), and, thus, results in higher thermostability under high light conditions.



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WHEN POPULATION GENETICS HIGHLIGHT THE EPIDEMIOLOGY OF GRAPEVINE DOWNY AND POWDERY MILDEW

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Molecular tools from population genetics and phylogenetics hold enormous promise for disease ecology because they enable the reconstruction of evolutionary relationships between pathogens on a wide range of spatial scales - from within host to between geographic locations. These approaches improve our ability to track pathogen movements, identify pathogen origins, predict evolutionary dynamics and understand environmental factors that influence their spread. In this line of investigation, we have performed a genetic study to highlight the population biology and the epidemiology of two important grapevine diseases, downy and powdery mildew.

Grape downy mildew is caused by the diploid *Plasmopara viticola* (Berk. & Curt. ex de Bary), a biotrofic oomycete native of North America where it attacks a large range of *Vitis* species. In the late 1870s, *P. viticola* was accidentally introduced into Europe, probably when American vine stocks resistant to grape phylloxera were used to graft the European varieties. Using nuclear and mitochondrial molecular markers (Delmotte et al. 2006, Chen et al. 2007, Giresse et al. 2009), we have studied the population genetic structure of *P. viticola* at varying spatial scales in order to address four specific questions. First, what is the level of genetic diversity of *P. viticola* in its native range of North America? Second, what level of genetic variability that has been introduced into France at the end of the 19th century? Third, can we detect spatial structure in the variability of the European populations of *P. viticola*, and if so, what are the biological consequences of this? We propose that the answers to these questions will allow us to evaluate the evolutionary potential of this plant pathogen species.

Grapevine powdery mildew, which is caused by the biotrophic ascomycete *Erysiphe necator*, provides a model system for investigating the effects of genetic structuring of plant pathogens on plant disease epidemics. *E. necator* populations are to exist in two genetically distinct groups (A and B) that have been identified in a number of grapevine-growing countries, including France, Australia, Italy and Spain. We have used a landscape population genetics approach to study the geographic distribution of genetic groups of *E. necator* across a large wine growing region of the south of France in order to assess the temporal succession of pathogen groups along the course of the epidemic. While the spatial distribution revealed a high level of heterogeneity between vineyards, a temporal succession of pathogen genotypes was observed in all populations: group A isolates tend to disappear during the course of the epidemic, whereas group B isolates are active during the entire growing season. We also demonstrate a strong positive correlation between disease severity and the genetic composition of *E. necator* populations, the damage being much more important when the epidemic was initiated by B isolates.

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EFFECT OF ENVIRONMENT AND GENOTYPE ON DISEASE SENSITIVITY

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Grapevine powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) are devastating pathogens, both of which are widely accepted to have originated in the eastern US and to have spread to Europe and around the world on planting material.

These pathosystems provide textbook examples of co-evolution - while the vast majority (>99.9%) of cultivars of the European grapevine *V. vinifera* are highly susceptible, many accessions of the co-evolved wild North American species are highly resistant. To-date, we have screened over 1400 accessions of wild and cultivated grapevine for resistance to each pathogen and have confirmed the presence of race-specific resistance using clonal isolates (Cadle-Davidson, 2008; Gadoury and Pearson, 1991). Thus, disease sensitivity is shaped by genetic diversity of the pathogen population, and we have begun to characterize the population genetics of *E. necator* in North America, Europe, and Australia. Not surprisingly, race-specificity and reduced efficacy of resistance have been particularly notable in regions with the most diverse pathogen populations, such as western New York State, where for example, local pathogen populations have adapted to the predominant host genotypes and related wild species (e.g., Concord, Niagara, and *V. labrusca*).

Environment also has profound interactions with disease sensitivity, even on dormant vines. Regions with protracted cold winters induce synchronous and rapid bloom wherein grapes complete anthesis in 48 to 72 hours. Consequently, berries synchronously acquire ontogenic resistance to a broad spectrum of fungal diseases. Insufficient midwinter chilling in warmer viticultural regions causes protracted and asynchronous bloom and an equally protracted period of berry susceptibility. We developed models to describe the development of ontogenic resistance in a broad range of climates, addressing a critical component of disease management, particularly in intermediate climates subject to shifts in the heterogeneity of grapevine phenology due to climate change.

Environmental factors during the growing season, both night and day, also impact disease sensitivity. For instance, the progress of powdery mildew epidemics is sensitive to low night-time temperatures, which can significantly reduce infection efficiency, colonization, latency, and conidiation on normally susceptible tissue. Sun exposure also reduces the severity of powdery mildew, and we have shown that this due both to the impact of UV radiation and increased daytime leaf temperature of exposed versus shaded leaves. Viticultural practices that aim to manipulate these factors advantageously can significantly reduce powdery mildew severity.

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THE GRAPEMILDEWS EXPERIMENT: SIGNPOSTING ONE PATH TO TREAT LESS AND ACCORDING TO EPIDEMICS AT THE PLOT LEVEL

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Grapevine powdery and Downy Mildews are the two most malignant pathogens of the vines (Aubertot et al., 2005). These diseases can potentially destroy the whole production each year. To protect their production, growers from Bordeaux apply on average 6 and 7 treatments against powdery mildew and downy mildew respectively (B. Léger, 2008, p. 239). However, there is some margin for progress and not all treatments made every year are useful. INRA's UMR Santé Végétale has come to the understanding that providing the growers with the knowledge necessary to reduce the number of spraying is not sufficient. It is necessary to show how to use that knowledge effectively (Clerjeau, 2004). Since 2001, the UMR is thus involved in the design of a low fungicide crop protection strategy through the use of state of the art epidemiological results and know-how nevertheless keeping in mind the growers economic constraints and aversion to risk (Clerjeau, 2000). The result of this research is GrapeMilDeWS (B. Léger et al., 2010). This crop protection guideline has been elicited and formalised using the Statechart modelling language. This work helped the pathologists to ameliorate their design through a more systematic approach of the decision logic. It provided a computer ready format of the process for integration into a simulator or a decision support system. Yet, the main purpose of GrapeMilDeWS' model is to provide an exhaustive specification of the decision process, which could then be transferred to other researchers and development workers (B. Léger, 2008). As of today, GrapeMilDeWS is being experimented in several wine producing regions of France (Delière, Cartolaro, Léger, Naud, & Ugaglia, 2009). These large scale experiments are necessary to create references in the behaviour of the pathosystem under low input management and for various bioclimatic conditions. GrapeMilDeWS is a learning tool for the growers, development worker and, still, for its original designers.

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LES NTIC, OUTILS POUR MAITRISER L'APPLICATION DES TRAITEMENTS PHYTOSANITAIRES ET LA TRACABILITÉ

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In France the concept of precision viticulture is a topical subject in relation with the "Grenelle environment" round table process. If the increase of productivity is one of its improvements, the reduction of the environmental impacts is another very important objective. New technologies of Information and Communication (NTIC) allow to respond to this challenge of quality. These technologies give solutions to manage pesticide applications tanks to the field work assistance tools and allow continuous monitoring and recording of the field operations to implement traceability and automatic field logbooks. This communication present the results of LIFE AWARE & TICSAD projects based on NTIC. We will approach the possible tracks allowing to manage plots variability.

Keywords: Vineyard, Precision Viticulture, Spraying, Embedded Equipment, Traceability.

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GRAPEVINE BREEDING FOR DOWNY MILDEW RESISTANCE

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A wide range of pathogens threatens viticulture. The current strategy to control grapevine diseases relies totally on the use of fungicides. This practice not only is 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 responsible to the chemical treatments. However *Vitis* species closely related to cultivated grapevine were already 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 caracterising 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. Moreover the results of the programmes carried out on the determinism of resistance to powdery mildew (Barker et al 2005), berry quality components (Duchêne et al 2009), sex (Marguerit et al 2009) and phenology are progressively integrated as well.

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DIVERSITY IN OIDIUM RESISTANCE REGIONS AND DIFFERENTIALLY EXPRESSED GENES

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Oidium (*Erysiphe necator*), the powdery mildew fungus of grape, is one of the major pathogens threatening viticulture. It was inadvertently introduced from North America to Europe in the mid 19th century, attacking leaves and fruits of the precious European *Vitis vinifera* grapevine cultivars. Resistance breeding is the only solution to reduce inevitable fungicide applications.

Breeding for fungal resistance relies on the introgression of resistance factors from *Vitis* wild species that co-evolved with the pathogen in its indigenous areas by experimental crosses. Recurrent back-crosses to *V. vinifera* are necessary to combine resistance with best wine quality. The introduction of genomic segments carrying resistance factors can now be monitored by the use of trait-linked molecular markers. These became available through genetic mapping studies performed during the last years (Barker et al., 2005; Akkurt et al., 2007; Welter et al., 2007).

However, the best trait-linked markers should be the resistance genes themselves. Analysing the expression patterns and sequence diversity of candidate genes should help to elaborate their function, regulation and hierarchical order during successful plant defense. In this context we studied genes differentially expressed during experimental inoculation of the resistant grapevine cultivar `Regent´ in comparison to a susceptible variety. The focus was laid on potential transcription factor genes whose expression pattern was investigated by quantitative Real Time-PCR. Currently, the sequence diversity of corresponding coding regions and putative promoters is under analysis. For this purpose a larger sample set of 45 *Vitis* genotypes exhibiting graded expression of Oidium resistance and different levels of genetic relationship to each other have been selected. Results from this work will be presented.

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BETA-AMINOBUTYRIC ACID-INDUCED RESISTANCE IN GRAPEVINE

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Grapevine (*Vitis vinifera* L.) is a major fruit crop worldwide and is affected by many diseases. Downy mildew, caused by the oomycete *Plasmopara viticola* is one of the most serious diseases in vineyards worldwide. Both susceptible and resistant cultivars can be colonised by *P. viticola* zoospores, but in resistant ones, the development of the parasite is rapidly inhibited. The majority of the traditional cultivars that are cultivated are susceptible to this disease, necessitating the intensive use of chemicals to limit the damage in vineyards. One possible solution would be the activation of the plants own defence system, known as induced resistance.

Beta-Aminobutyric acid (BABA), a non-protein amino acid, has previously been shown to induce resistance against many oomycetes and to be effective in inducing resistance against various downy mildews. It was observed that the protective effect of BABA in Arabidopsis was due to the potentiation of natural defence mechanisms, a phenomenon referred to as priming. Priming is the capacity of a plant to express a faster and stronger basal defence response upon pathogen infection. Recently, in grapevine it has been shown that callose deposition as well as defence mechanisms depending on the phenylpropanoid and the jasmonic acid (JA) pathways all contributed to BABA-IR in the susceptible cultivar Chasselas. Microarray analysis was performed to compare gene expression in BABA-and water-treated infected Chasselas (susceptible cultivar).

BABA can also prime resistance of grapevine to abiotic stress. BABA-treated leaves close their stomata faster upon drought stress, probably via an increased ABA production. Microarray experiments also reveal a number of induced and repressed genes that could contribute to this better adaptation.

The expression of a small number of genes is modified by BABA but a higher number is primed both in pathogen-defence and in drought adaptation

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REDUCTION OF GRAY MOLD IN GRAPES BY ETHANOL APPLICATIONS

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Pre-harvest applications of a 16% ethanol (EtOH) solution, containing 1 % of calcium chloride ($CaCl_2$), reduced gray mold development in 'Chasselas' table grapes picked at a late harvest date, the losses due to rotten clusters dropped from 15% in controls to 5% in grapes treated with EtOH+$CaCl_2$. Then over a 6-week cold storage, the losses due to gray mold rots were reduced by 50% when storing EtOH+$CaCl_2$ treated clusters, compared to untreated controls. Preliminary experiments had shown that a 2% EtOH solution was already inducing significant drop of gray mold growth. A range of concentrations up to 50% ethanol had been tested in preliminary trials without observing damages to the vines and clusters. The treatments did not induce significant changes to the fruit quality assessed by sensory analyses on healthy berries.

Post-harvest application of ethanol vapours has been optimised over two seasons in order to prevent rot development, caused by *Botrytis cinerea*, and stem browning in 'Chasselas' table grapes. At a dose rate of 2ml kg−1 of grapes, ethanol vapour was as effective as sulphur dioxide pads. Consumer panels detected no significant difference in sensory perception between controls and treated grapes. The ethanol vapour treatment could be easily implemented by the table grape industry since the technology is similar to sulphur dioxide treatment.

A combination of both pre- and post-harvest treatments has not been tested yet.

BURKHOLDERIA PHYTOFIRMANS STRAIN *PSJN* PRIMED THE EXPRESSION OF STRESS-RELATED GENES IN *VITIS VINIFERA* L. UPON LOW NON-FREEZING TEMPERATURES

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Key words: priming, cold stress, PGPR, PsJN, defense gene, Cbf4, grapevine.

Burkholderia phytofirmans strain PsJN is a plant growth promoting rhizobacterium (PGPR) able to establish rhizospheric and endophytic populations in various crops. In grapevine, this bacterium stimulates growth and induces physiological changes leading to an increase of plant resistance towards cold stress. In this study, we further analyzed the effects of bacterization of *in vitro* grapevine plantlets when exposed to low non-freezing temperatures. In this aim, we followed by quantitative RT-PCR the expression of various stress-related genes coding for stilbene synthase (*VvSTS*), phenylalanine ammonia-lyase (*VvPAL*), lipoxygenase (*VvLox*), 3 pathogenesis-related proteins (*VvGluc*, ß-1,3-glucanase; *VvChit4c*, chitinase; *VvChit1b*, chitinase) and the transcription factor CBF4 (*VvCBF4*) known as cold marker in grapevine.

Our results showed a faster and stronger gene expression in bacterized plantlets compared to non-bacterized plantlets at 4°C for all studied genes. This state was maintained 2 weeks after cold stress. This indicates that *B. phytofirmans* strain PsJN induces in grapevine a primed state that helps the plant to overcome cold stress.

COVER CROPPING AND DEFICIT IRRIGATION STRATEGIES: EFFECTS ON WATER RELATIONS, GROWTH AND FRUIT COMPOSITION IN TEMPRANILLO GRAPEVINES

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Considering the predicted scenarios of climate change over the next decades, Mediterranean vineyards will face a substantial shift in precipitation pattern. Adaptation of cultural practices are needed in order to mitigate the negative effects on yield and wine quality. Deficit irrigation strategies combined with cover crops are one of such tools. In dry viticultural regions deficit irrigation strategies such as regulated deficit irrigation (RDI) and partial rootzone drying (PRD) have been successfully adopted to control vine vegetative growth and improve fruit composition, while enhancing plant water use efficiency. In Mediterranean climates, where accumulated Winter and Spring rainfall can induce high soil water at bloom time, the use of cover crops combined with RDI are valuable tools to control vegetative growth and berry size. Indeed, the additional water used by the swards in Spring can remove excess water from the root zone, generating a desirable mild water deficits. With the aim to test the effects of vineyard floor management practices combined with deficit irrigation strategies an experiment was set up in a commercial irrigated Aragonez (syn. Tempranillo) vineyard located in South Portugal. In a split-plot factorial design two floor management practices (soil tillage - ST - and permanent resident vegetation - RV) combined with three deficit irrigation strategies (RDI, PRD and DI, conventional deficit irrigation) were studied. The irrigation started after full bloom for PRD and DI, and two weeks later for RDI. The annual broad-leaved species comprised the majority of the plant species surveyed in both floor treatments. After the mowing and soil cultivation carried out at the end of April, RV treatment showed a significantly higher total amount of above-ground dry matter than ST until the end of Spring. Compared to soil tillage the resident vegetation was effective in reducing soil water content during the Spring, inducing a significant reduction on vine vegetative growth, berry weight, yield and titratable acidity. As for the irrigation treatments we observed that RDI induced a reduction in vine vegetative growth, berry weight, yield and titratable acidity when compared to PRD and DI, which presented similar results. Our work also showed that in low vigour vineyards and with low irrigation amounts (as was the case in the present experiment), PRD was unable to induce better agronomical results than DI, as we previously observed in other combination of climate, soil and varieties. We therefore conclude that, for the ecological and viticultural conditions of our experiment, DI should be preferred to RDI and PRD as it is the simplest deficit irrigation strategy, still enabling an efficient control of vegetative growth without negative impact on yield and berry composition as compared to RDI. Moreover, the degree of water competition between the cover crop and the vines must be carefully managed. The very high sensitivity of shoot growth to early water deficits makes the onset of irrigation in RV a crucial decision in order to avoid an incomplete canopy establishment like it was the case of the current study.

WATER STRESS MANAGEMENT IN 'TEMPRANILLO' VINEYARDS AIMING AT HIGH QUALITY. INTEREST OF DEFICIT IRRIGATION STRATEGIES

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Regulated Deficit Irrigation (RDI) is an irrigation scheduling technique originally developed for fruit orchards that has been successfully adapted for winegrape production. The aim of this work is to present the results of a 4-year experiment comparing two Regulated Deficit Irrigation (RDI) strategies with conventional irrigation practices (CI). CI consisted in a progressive increase in water deficit as summer progressed, whereas RDI strategies (RDI1 and RDI2) had in common a deficit period just after fruit set, and in RDI2 treatment vines were subjected to an additional stress period just after veraison. The experiments took place from 2003 to 2006 in a commercial vineyard located in Traibuenas, Southern Navarre (Spain), with a typical Mediterranean climate, with rainfall below 400 mm. Both deficit irrigation strategies allowed to control vine vigor and to obtain moderate yields, since the water stress elicited during berry green development stage reduces both final berry size and cluster differentiation for the following year (less and smaller clusters). No relevant differences in sugar concentration were found, probably due to the fact that rainfall in the last period of ripening permitted a recovery of photosynthetic activity in all the treatments. RDI berries tended to have lower acidity but the most relevant effect of RDI strategies on grape quality was an increase in anthocyanin and phenolics concentration. In RDI1 berries this increase was just an indirect consequence of smaller berry size. However, in RDI2, anthocyanin gain cannot be explained completely this way, so the second deficit period somehow contributes to promote synthesis and/or translocation of anthocyanins. Since harvest quality has been clearly improved by any of the RDI strategies in both years, despite ripening periods were much more rainy than average, it can be concluded that RDI constitutes an interesting technique to be applied in 'Tempranillo' vineyards grown in semiarid areas aiming to obtain high quality grapes.

IDENTIFICATION OF WINE *VITIS VINIFERA* GENOTYPES, AUTOCHTHONOUS TO CRETE, GREECE, EMPLOYING AMPELOGRAPHIC, AFLP AND SSR MARKERS

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Fifty three biotypes (candidate clones) belonging to seven different traditional *Vitis vinifera* cultivars, autochthonous to the island of Crete, Greece were selected and classified by ampelographic descriptors. Subsequently, in order to solve accession labelling problems, characterize genetic diversity, and establish genetic relationships within and between cultivars, biotypes were treated as independent genotypes and were fingerprinted employing two types of molecular markers; Amplified Fragment Length Polymorphisms (AFLP) and microsatellites (SSR). Four of the cultivars namely Vidiano, Vilana, Plyto, and Moschato Spinas are white-wine producers while the rest, namely Kotsifali, Liatico and Mantilari are red-wine producers. Two white-wine producing cultivars originating from outside Crete, namely Moschato Samou and Moschato Alexandrias were used as out-group controls. Four AFLP primer combinations were employed yielding 51 polymorphic AFLP markers which in turn allowed for the grouping of the majority of individual genotypes, for 5 of the examined cultivars, within single clusters -specific to each cultivar. On the other hand, approximately half of the total individual genotypes (mostly from white wine cultivars) remained unclustered. Fifty seven (57) SSR alleles were amplified from 6 SSR loci across all employed genotypes. When compared to AFLP, SSR markers offered lower individual genotype resolution within cultivars but grouped the majority of individual genotypes with the anticipated cultivar cluster. For 29 of the examined genotypes at least one unique SSR allele was detected. The overall observed heterozygosity was 0.76. In general, partial agreement was found between ampelographic descriptors and AFLP markers owing to the low clustering resolution of the later while very good agreement was found between ampelographic descriptors and SSR markers. In future work, use of greater number of molecular markers could increase the agreement between the three types of classification system employed.

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GENETIC AND CHEMICAL INVESTIGATION OF SWISS GRAPE VARIETIES

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During the set up of the online Swiss Vitis Microsatellite Database (SVMD), we noted that more than 100 grape varieties are commonly cultivated on the 15' 000Ha of vineyards of Switzerland. The SVMD gathers 103 cultivated types of vines, 20 interspecific hybrids, 16 rootstocks and 32 vines genotypes with 6 microsatellites. With the increasing interest of the public and the wine industry in ancient traditional varieties, we started to investigate unknown parentage and other genetic relationships of autochthonous grape varieties (including wild grapevines) from Switzerland with up to 60SSRs. The results of our studies find issues in the marketing of Swiss wine industry and provide fundamental information for grape breeders and researchers working on grape quality improvement and resistance.

POSTERS

P1

SCREENING PHYTOLAEXINS IN STEM BARK OF SWISS VITIS VINIFERA CULTIVARS

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Phytoalexins have been shown to possess biological activity against a wide range of pathogens and can be considered as markers for plant resistance. Phytoalexins form the Vitaceae is constituted of a restricted group of molecules belonging to the stilbenes family, the skeleton of which is based on trans-resveratrol structure. Several simple stilbenes such as *trans*-pterostilbene (dimethylated resveratrol), *trans* and *cis* piceid a 3-O-α-D-glucoside of resveratrol, and oligomers of resveratrol as viniferins have also been found in grapevine as a result of infection or stress. The major compound appears to be ε-viniferin, a cyclic dehydromer of resveratrol.

Grapevine wood diseases such as eutypa dieback, esca, and black dead arm, are destructive diseases affecting vineyards all over the world, caused by one or several fungal xylotrophs respectively. The main research findings concern pathogen identification, reproduction of symptoms through inoculation with various fungi, as well, the influence of the environment on the incidence of the disease was studied. There is no emphasis on the influence of the wood constituent such as phytoalexins on fungal growth or diseases development within the bark.

The aim of this study was to screen the wood of *V. vinifera* cultivars for the presence of phytoalexins in order to establish a correlation between the abundance of phytoalexins and the resistance of the cultivar to wood pathogens. Twenty swiss red and twenty eight white cutivars were selected for this study. Phytoalexins were extracted from basal stem bark of healthy plants. One step extraction allowed the extraction of 87 % of the phytoalexins. Samples were analysed by riverse-phase chromatography with diode array detection. Quantification of phytoalexins was performed by adding an internal standard, and identification by comparison of retention and UV spectra with the standards. Standards were preliminary isolated and identified by MS and RMN analysis from grape stem bark. We report, the quantification of *trans* and *cis* piceid, *trans* and *cis* resveratrol, *tran*s and *cis* ε-vinifeine, *trans* and *cis* vitisine A.

Finally a hypothesis of the intrinsic genetic factors of *V. vinifera* in relation of phytoalexin biosynthesis and resistane and/or tolerance against pathogen is drawn.

P2

BIOPROTEC
A TECHNICAL PLATFORM FOR BIOPESTICIDES DEVELOPMENT

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Nowadays, more than 75 000 tonnes per year of pesticides are used in France for crop protection, France being the first consumer in Europe.
Many studies show the effects of these chemical products on human health and environment. In order to adhere to the objectives defined by the "Grenelle de l'environnement", which envisages to reduce by 50% (if possible) the use of pesticides (Plan Ecophyto 2018), the Bordeaux Montesquieu Association considers the creation of a platform (BIOPROTEC) to support the development and the use of biological agents (biopesticides) as crop protection alternative solutions.
These natural products, more respectful of the environment and the health of the users and the consumers, could also be introduced into traditional chemical treatment programs in order to decrease the use of pesticides.
This platform will be bound for research laboratories and private companies and will aim to promote the appearance of new natural products on the market. It will be based on already existing scientific competences network implied in the development of biological agents for crop protection.
The platform will thus enable to structure this biopesticides network and to promote the emergence and the achievement of projects. It will also provide an integrated offer of research and development services. The advantage of the creation of such a structure is to be able to bring a global offer helping the various users at all the development levels of crop protection natural products.

P3

GROWTH AND GENE EXPRESSION RESPONSE OF GRAPEVINE GENOTYPES UNDER OSMOTIC STRESS.

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Like all other important crop species, vineyards are expected to face to the effects of climate change. An alternative and sustainable strategy to overcome the problem of limited water supply rely on plant material. In a large majority of vineyards, vines are grafted and a vine is consequently a combination between two genotypes, a rootstock and a scion, both potentially contributing to the response of the vine to altered environments. Characterization of gene regulation is fundamental for achieving an understanding of the complex processes for biotic and abiotic stress. Up to now, it is still unknown how many genes are involved in osmotic stress in grapevine (*Vitis*). In this study, we examined the gene expression of three different contrasting genotypes: Cabernet Sauvignon (*Vitis vinifera*), Riparia Gloire de Montpellier (*V. riparia*) and 110 Richter (*V. rupestris* x *V. berlandieri* hybrid). All these three genotypes were used as rootstock and grafted with Cabernet Sauvignon cultivar (*V. vinifera*).
One year old grafted plants were grown in aeroponic conditions with aerated nutrient solution under greenhouse controlled conditions. After 2 months of growth,, PEG treatment was applied by adding to the same nutrient solution 0,5% and 1,25% PEG (molecular weight 6000). Normal irrigation was maintained for control plants. For the examination of stress-responsive genes, RNA was isolated from grapevine roots at 1 h, 6 h, 24 h and 7 days after the stress treatment. Differential regulation of selected genes was investigated by performing real-time RT-PCR analysis from three biological replicates. Results indicated that plant response varied between genotypes after stress application. Modifications of plant growth were observed between the three genotypes and time-course analysis of transcript accumulation indicated that the expression of root-specific genes changed during stress, which were probably associated with adaptation/response mechanisms. The results that will be presented provide information on gene-encoding factors implicated in drought tolerance.

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P4

INFLUENCE OF MICROCLIMATE CONDITIONS ON PROTEIN EXPRESSION IN GRAPE BERRY

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Climate change has the potential to greatly impact grapevine culture modifying phenological timing and hence, metabolic composition in grapes and wine (1,2). In order to improve the knowledge on the impact of the microclimate on grape berries, we studied changes in the proteome of field grown sun-exposed or shaded berries on the east or west side of the row and inside or outside the canopy. The comparative proteome analysis from whole berries by 2-D PAGE (905 total proteins) identified 86 proteins showing differential abundance as a response to microclimate changes. The largest functional classes comprised proteins involved in glycolysis, Krebs cycle, photosynthesis, carbohydrate metabolism, respiration and energy. A high proportion of heat shock proteins (25%) was differentially expressed in agreement with their expected role in stress induced by temperature. Also, numerous proteases (10%) and several proteins related to secondary metabolism, cell structure or protein regulation as well as proteins with unknown functions were identified. These results give new insights to the berry proteome and showed that it might be significantly affected by microclimate changes.

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P5

PROTEOMICS AS A TOOL TO UNDERSTAND THE PHYSIOLOGICAL STATE OF GRAPEVINE DURING PLANT- PATHOGEN INTERACTIONS

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The cultivated grapevine (*Vitis vinifera* L.*)* is a significant contributor to the total economic value of agricultural production worldwide. Under field conditions, grapevine is exposed to attack from a variety of pathogenic fungi and other pests, which are associated with the occurrence of diseases, such as powdery mildew, downy mildew, eutypa dieback and esca (Ferreira *et al.,* 2004). The occurrence of disease is associated with modulation of plant defence proteins, as well as that of fungal virulence enzymes. The former plays a crucial role in host defence, by synthesising plant defence metabolites and by being active in the degradation of fungal cell walls. The latter may have a function in the detoxification of plant defence metabolites.

We present here an overview of the changes occurring in the grapevine proteome following the onset of disease. Several pathosystems are being investigated, which highlight the importance of protein modulation in plant defence and in the study of plant-pathogen interactions.

In the first instance, the role of dirigent proteins has been evaluated in grapevine affected by powdery mildew. These proteins are responsible for the 'stereospecific direction' of the synthesis of lignin and lignans (Davin & Lewis, 2000), which are important structural and biochemical defence mechanisms adopted by plants to counteract biotic stress.

The second example relates to the role of tannin polymerase in the detoxification of tannins that may interfere with or inhibit fungal growth. By evaluating the role of this enzyme on the fungus responsible for powdery mildew, we intend to highlight perhaps one of the principal mechanisms responsible for the widespread occurrence of this disease in grapevine.

Finally, we present some of the initial results relating to the proteomic changes occurring following the onset of Petri disease, which indicate a shift in the proteome brought upon by the disease. Protein extraction from plant woody tissues poses considerable challenges, the main one being the limited amount of protein encountered in this type of tissues. Such challenges are enhanced when working with grapevine tissues, which are particularly rich in phenolic compounds.

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P6

UNRAVELLING THE FUNCTION OF GRAPE FLAVONOID REGULATORS BY OVEREXPRESSION IN HETEROLOGOUS SYSTEMS

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Anthocyanins are accumulated during ripening of grape berries and represent the main source of pigment in wine. Besides the well characterized regulative role of the transcription factor VvMYBA1, the co-regulation of anthocyanin pathway by other members of the MYB family (VvMYB5a and VvMYB5b) has been proposed. The role of VvMYB5a and VvMYB5b has been mainly inferred by expression analyses and/or ectopic expression in heterologous systems.

We used functional complementation analyses of the well characterized Petunia anthocyanin regulatory mutants to gain information about the role of VvMYB5a, VvMYB5b and VvMYBA1 in the regulatory network operating in *Vitis vinifera*.

In petunia the mutation of ortholog of *VvMYB5a* and *VvMYB5b (PhPH4*) and of *VvMYBA1 (PhAN2)* result in the increase of vacuolar pH and strong reduction of anthocyanin content of petals respectively. The coding sequence of VvMYB5a, VvMYB5b and VvMYBA1 was fused to the constitutive promoter 35S and transformed into *ph4* and *an2* petunia mutant lines. Analyses of transgenic plants revealed full complementation phenotypes. A deep characterization of pigment profile, vacuolar pH and expression of structural genes confirmed that restored phenotypes were attributable to an activation of target genes belonging to vacuolar acidification and anthocyanin pathways. Moreover, specific and/or partially overlapping effects could be observed, giving insights about their possible redundant roles in grape.

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P7

ETHYLENE SIGNALLING MEDIATORS OVER THE GRAPE BERRY DEVELOPMENT: GENE EXPRESSION PROFILING

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The ethylene signalling pathway has never been fully described in grapes. Regarded as a non-climacteric fruit, grape berry seems to ripen independently to ethylene, however 1-methylcyclopropene (1-MCP), a specific inhibitor of ethylene receptors has been shown to alter berry ripening processes. Here, we report profiles of transcript abundance of various mediators, associated with ethylene signalling, throughout berry development. For instance, mRNAs of *VvETR2* (ortholog to *AtETR2*) showed a transient peak at the inception of ripening in Cabernet Sauvignon berries coinciding with an internal ethylene peak, prior to colour changes. The transcripts of other orthologs such as *VvRTE1* and *VvEIN4* steadily increased over the berry development, while *VvERS1* ortholog transcripts exhibited a peak of accumulation only when the berries were fully coloured. Finally, mRNAs of two transcription factors, *VvEIN3* and *VvMADS4*, showed strong accumulation during the late phase of berry ripening. We also observed inflections of mRNA accumulation after incubating berry clusters with ethylene and 1-MCP. The main effect was observed with *VvEIN3* transcripts that showed a significant up-regulation after incubation with 1-MCP. Furthermore, other transcript levels (*VvETR2* and *VvCTR1*) were also increased by exogenous ethylene, once the colour change was initiated (*i.e.* 10 to 11 weeks after bloom). Some studies have already indicated that non-climacteric fruits shared signalling pathways with climacteric fruits. However, most differences between these ripening classifications remain undescribed at the genetic/molecular level. This first data set will allow us to better understand potential involvements of ethylene signalling in a non climacteric fruit such as grape berry.

P8
SPATIO-TEMPORAL STUDY OF DEFENSE GENES IN GRAPEVINE

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Pathogenesis-related (PR) proteins are important elements of the plant defense machinery. In grapevine (*V.vinifera* L. cv Pinot Noir) previous studies have shown that a chitinase (CHV5) and a thaumatin-like protein (TL) accumulate in berries during fruit maturation and represent the two most abundant extractable proteins of ripe berries (Derckel *et al.* 1998 ; Manteau 2003). The aim of this work is to investigate how the expression of both PR-proteins is affected by abiotic and biotic stresses. We showed that CHV5 and TL are induced in leaves and young berries by UV-C irradiation as well as in leaves during *Botrytis cinerea* infection. These results confirm that CHV5 and TL are referred to as PR-proteins. On the opposite in ripe berries collected in vineyard CHV5 and TL proteins decreased as the infection by *Botrytis cinerea* develops. This necrotrophic fungus was then supposed to degrade these proteins. In addition CHV5 and TL mRNAs also decreased during this infection process, suggesting that another mechanism is involved. To investigate the molecular interactions between *B. cinerea* and *V. vinifera* and to explain proteins degradation and mRNAs decrease, we studied the tissular localization expression of both PR-proteins by *in situ* hybridization and immunolocalization. The first results showed that after UV-C irradiation of young berries, TL mRNAs accumulated around vascular bundles in the pulp and the exocarp and CHV5 mRNAs mainly around proximal vessels. Immunolocalization studies showed that CHV5 proteins localized in the epicarp as well as around the vascular bundles, suggesting that the anti-CHV5 antibody used may bind to several isoforms. Localization of mRNAs/proteins sites in *B. cinerea*-infected berries is under investigation. In addition, proteins production by heterologous system (in progress) will allow us to characterize these two PR-proteins and to better understand the mechanisms of interaction between *B. cinerea* and *V. vinifera* PR-proteins.

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P9

HOW DIFFERENT ARE *VITIS VINIFERA* GENOTYPES IN PHYSIOLOGICAL RESPONSES DURING PROGRESSIVE DROUGHT AND RECOVERY?

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Drought is one of the most important abiotic stress factors affecting grapevines under Mediterranean and arid climate conditions and it is usually accompanied by heat stress. This has negative consequences for vine's growth and berry quality. Therefore judicious irrigation and increased water use efficiency (WUE) remain major research topics in the context of irrigated viticulture (Costa et al., 2007; Chaves et al., 2007). The large genetic variability among *Vitis vinifera* varieties results in large differences in the response to water stress. Genotype related differences in WUE are linked to variation in leaf gas-exchange because net photosynthesis, stomatal conductance and WUE were shown to vary with the genotype (Chaves et al., 1987; Bota et al., 2001; Costa et al., 2008). However, variation in photosynthetic characteristics among varieties seems less relevant than diversity in stomatal regulation (Bota et al., 2001). Our field studies showed differences for leaf gas-exchange and intrinsic WUE traits among a group of five red grape varieties grown under deficit irrigation, in particular, between Syrah (SYR) and the Portuguese variety Touriga Nacional (TOU), both grafted on the 1103-P rootstock (Costa et al., 2008). Further studies were carried out under greenhouse conditions to assess response of these two cultivars to severe or moderate water stress and recovery. Observations were also done for plants of the rootstock 1103-P and TOU on own roots. Plants were grown in pots and were subjected to two treatments: fully irrigated and non-irrigated. Water was arrested for a period of 16 days, after which plants were again fully irrigated. Measurements took place during the drought period and the week after irrigation has restarted. Pre-dawn leaf water potential was measured every 3 to 4 days. Leaf stomatal conductance, net photosynthesis and PSII efficiency were measured periodically under conditions of saturated light (1200 $\mu molm^{-2}s^{-1}$), temperature of 29°C and constant air CO_2 by using a portable photosynthesis system LI-6400 (Li-Cor,USA) equipped with a fluorescence chamber. Xylem sap was also extracted to quantify ABA content in the different cultivars. Specific leaf area, leaf stomatal density and anatomy and Chl content were determined. . Measurements were done in August 2008 and 2009. Results will be presented and discussed with respect to both scale and time course of the responses of the different genotypes and will be compared with field studies.

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P10
MODELLING APPROACHES TO PROVIDE NOVEL INSIGHTS INTO THE COMPLEX REGULATION OF GRAPE BERRY GROWTH AND QUALITY

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Process-based models can mathematically integrate as many as possible physiological processes involved in defining fruit growth and composition, and quantify plant response to environmental factors and management practices, making them a promising tool to evaluate the combined effects of several factors (Struik et al. 2005). So far for grapevine, there are many models focusing on shoot growth, canopy structure, response to pruning, water balance at the vineyard level, and dry mass accumulation and allocation at the canopy level ; but process-based models for fruit quality and growth are rare (Génard et al. 2007).
Here we present our pionner work in modelling berry quality. A biophysical growth model originally designed for peach (Fishman and Génard 1998) was successfully adapted to grape (Dai et al. 2008). Coupled with a sugar accumulation sub-model (Génard and Souty 1996), it can simulate the effects of various leaf-to-fruit ratios on fresh and dry mass accumulation in a ripening berry, and can quantify the relative contributions of sugar import, sugar metabolism, and water budget to the responses of sugar concentration to assimilates and water supply (Dai et al. 2009). Our results give possible interpretations about adaptation of grapes to stresses. The model could provide valuable framework to simulate the complex behavior of fleshy fruits to climate change, which in grapevine will impact berry metabolism directly (by modifying berry microclimate) and indirectly (by altering water and sugar fluxes entering the berry).

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P11
COMPARISON BY PROTEOMIC APPROACH OF PROTECTIVE AND NON PROTECTIVE ELICITORS IN *VITIS VINIFERA* AGAINST *BOTRYTIS CINEREA*

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Key words : elicitors, protection markers, proteomic analysis, *Vitis vinifera*, *Botrytis cinerea*

Grey mold caused by *Botrytis cinerea* infection is one of the main diseases affecting vineyard. Unfortunately, the use of chemical treatments known to damage environnement, remains nowadays the main solution to cope with *Botrytis cinerea*, the agent of grey mold. An alternative strategy consists in using elicitors to stimulate plant defense mechanisms and thus prevent disease. Nevertheless no elicitors has shown to date a real protective effect against grey mold in the vineyards although most of them induce the expression of defense genes in controlled experiments. In this context, it appears crucial to characterize biomarkers which would enable to discriminate between grapevine defense stimulation and effective protection against *B. cinerea*. The aim of this study is to unravel protection biomarkers by two dimentionnal electrophoresis proteomic analysis comparing the effects of « protective elicitors » with those of « non protective elicitors ». Our first results allowed to identify three different elicitors with protective or non protective activity against *B. cinerea*. Previous data shown clear differences between this three elicitors in their protein expression profiles. Further experiments should allow to characterize specific protection biomarkers in grapevine against *B. cinerea.* The identification of protection biomarkers would improve comprehension of plant defense mechanisms against *Botrytis cinerea* and allow to developp large scale screening tools for analysis of new elicitors confering grapevine protection against grey mold and other pathogens.

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P12
TRANSCRIPTIONAL PROFILING OF LATENT BUD DEVELOPMENT

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Crucial grapevine developmental processes take place within developing buds along consecutive growing seasons. The most relevant one is flowering induction, which takes place in early summer latent buds and causes the differentiation of lateral meristems into inflorescence meristems. Subsequently, branching of the inflorescence meristem, that gives rise to inflorescence branch meristems, progresses until the beginning of the dormancy period at the end of the summer. The following season, after dormancy, bud development resumes and more inflorescence branch meristems are formed before each one differentiates into a cluster of 3 to 4 flower meristems that will give rise to flowers.

To get insights into these developmental processes, we have performed a transcriptomic analysis along different stages of bud development using the Grapegen gene-chip. This Affymetrix GeneChip contains 23000 probe sets representing approximately 50% of the annotated genes in the grapevine genome. As a first approach, we have analysed transcription profiles of latent buds of Tempranillo monthly collected during two years from May of the first year to April of the second season.

The results indicate the existence of two main expression profiles; the first one corresponds to genes overexpressed in actively growing buds during the spring and early summer which expression get down with the summer dormancy entrance. These genes are involved in basic metabolic processes such as photosynthesis, electron transport, nucleic acid metabolism, and protein biosynthesis among others. The second expression profile corresponds to genes which expression is repressed in spring buds but are overexpressed with the initiation of dormancy. These genes generally fall in groups involved in transcriptional regulation, phytohormones synthesis and response as well as responses to several types of stresses. A more restricted analysis focused on transcription factors detects parallel changes in the members of this functional group, probably reflecting its involvement in dormancy regulation.

P13

GENETIC VARIABILITY OF WATER USE EFFICIENCY IN GRAPEVINE

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The variability on water use efficiency was evaluated in a collection of 22 grapevine cultivars growing in an experimental farm near Palma. Gas exchange parameters (net photosynthesis, stomatal conductance, transpiration) were measured in leaves four times from May to August. Also, water relation parameters (soil water potential, stem water potential), and grape yield and quality were also analyzed. The results shown that intrinsic water use efficiency (relation between net photosynthesis and stomatal conductance, WUE) measured in grapevine ranged from 42 to 78 μmol mol^{-1} in well watered plants, but mostly of the cultivars shown values around 60 μmol mol^{-1}. However when water deficit was progressively imposed, WUE increased and finally, raised values up to 150 μmol mol^{-1}. Under those conditions of very low soil water availability (soil water potential of -1,5 MPa), the WUE ranged from 72 μmol mol^{-1} (Macabeo cultivar) to 156 μmol mol^{-1} (Argamusa). The plant water status measured as stem water potential (Ψstem) under severe water stress conditions, ranged from -0,97 to -1,67 MPa, depending of the cultivar. Interestingly, Macabeo cultivar showed the lowest WUE and the highest Ψstem (-.0, 975 MPa). Also this cultivar presented the highest yield (fruit production per plant). On the opposite, Argamusa was the cultivar with highest WUE under water stress, because of a higher stomatal adjustment under those conditions, maintaining high net photosynthesis rates. This cvar also showed a very low stem water potential (-1, 48 MPa). The higher capacity of carbon fixation of this cultivar under water stress, was reflected in a high plant yield (7,8 Kg grape per plant), however sugar concentration in must was very low.

P14
MANAGEMENT OF BOTRYTIS BUNCH ROT CAUSED BY *BOTRYTIS CINEREA*

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Since a few years off-flavours have occurred in wines of Luxembourg. These off-flavours are the results of infections of grape clusters caused by different phytopathogenic fungi, especially *Botrytis cinerea* and *Penicillium expansum*. Because of the compact structure of their clusters, primarily wines produced out of varieties belonging to the „Pinot family“ were highly affected. To reduce this problem, different trials in practical and experimental vineyards were conducted in the years 2007 to 2009. More precisely, the effect of (a) early defoliation of the cluster zone, (b) the application of bioregulators (a.i. prohexadione-Ca, gibberellic acid), (c) the application of botryticides (a.i. fenhexamid, boscalid) and different combinations of these three measures were compared to the untreated control and to each other.

In the studied varieties (Pinot blanc, Pinot gris, Pinot noir), all these measures were able to reduce the disease severity of *Botrytis cinerea* as compared to the untreated control. The success of the three measures was comparable to each other with some variations between the years. Best results were, however, achieved through an early defoliation close to the bloom combined with the use of a bioregulator or a botryticide.

Up to now, it can be concluded that all these options (defoliation, bioregulators, botryticides) are able to reduce the risk of massive attacks by *Botrytis cinerea*, *Penicillium expansum* and sour rot. The combinations of two or all three measures increase the efficiency of the protective strategy. Further investigations will be done to schedule the moment for the partly defoliation of the cluster-zone and to optimize the application of bioregulators by clarifying their modes of action.

P15

A GENE EXPRESSION MAP OF *VITIS VINIFERA* CV. CORVINA DEVELOPMENT

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Transcriptional programs are important in the development of multicellular organisms. In contrast to most animals, plants develop continuously, with new organs being initiated and elaborated throughout the life cycle of the organism. As a consequence, individuals consist of repeated units, which are present in many developmental stages at any given time of the life cycle. It follows that many transcriptional programs underlying the development of different organ systems are continuously active. Plants therefore provide an opportunity to study how transcriptional programs control multicellular development. We analyzed global gene expression during development of the plant *Vitis vinifera* cv. Corvina in samples covering many stages and diverse organs. In particular, we obtained triplicate expression estimates from 25 samples, consisting of different stages of development of leaves, inflorescences, buds, berries, seeds, tendrils, stems and roots. We used NimbleGen 12x135K arrays, which contains 12 x 135,000 probe sets and enables to hybridize up 12 independent samples on a single slide. The aim of the project is to observe the expression levels of transcriptional factor genes and signal transduction components, comparing with those of metabolic genes. Moreover, it will explain if specialized expression pattern could be caused by preferential use of entire gene families in specific developmental processes or tissue-specific responses to the environment.

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P16

METHYL JASMONATE OR ETHEPHON TREATMENT *VERSUS* A CO-TREATMENT WITH BOTH MOLECULES ON GRAPEVINES: DEFENSE RESPONSES AND PROTECTION AGAINST *ERYSIPHE NECATOR*

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Powdery mildew, a grapevine disease caused by the biotrophic fungus *Erysiphe necator*, affects yield and vine fruit quality. The year 2008 was particularly marked by a high infection rate in France. Disease control is currently achieved by intensive use of fungicides. Owing to the « Grenelle Environnement » and the emergence of strobilurin resistance, wine grower and scientists are developing alternative approaches. One of these consists of inducing natural plant defenses by using elicitors. To date, only one elicitor has provisional marketing authorization: Stifénia® from Soft, but it remains inefficient in the case of high disease intensity. Based on these statements, we choose to develop new SDN (inducers of natural defenses) for the Vine. We are interested in two phytohormones involved in the signal transduction cascades leading to defense responses: jasmonic acid (its more active derivative form: methyl jasmonate) and ethylene (a precursor form: Ethephon). These molecules have been used as inducers of defense mechanisms in a number of systems. Experiments on Cabernet Sauvignon foliar cuttings shown that both molecules are able to trigger grapevine defenses (Belhadj *et al*. 2006 and 2008). On the one hand, by enhancing stilbene biosynthesis, the grapevine phytoalexins, and on the other hand by up-regulating about PR (pathogenesis related) protein gene expression. Correlated to this induction, methyl jasmonate or ethylene treatment alone triggers protection of grapevines against powdery mildew. In order to improve plant defense responses, a co-treatment methyl jasmonate/Ethephon was done (Faurie *et al*. 2009). This co-treatment leads to a synergistic action of both molecules on phytoalexin production. However, it inhibits PR protein gene expression and does not afford an increased resistance against *Erysiphe necator*. These results can be exploited for the development of new pest control strategies in the vineyard and underline the importance of understanding defense mechanisms for disease control in vineyards.

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P17

GRAPEVINE BUD DEVELOPMENT AND DORMANCY RELATED TRANSCRIPTOME

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Daylength is one of the important environmental cues synchronizing seasonal growth cessation and dormancy induction. Bud dormancy in grapevines is an adaptive strategy for stress survival, but also limits the range of cultivar adaptation. The influence of photoperiod treatments (long, 15h = LD and short, 13h = SD) on latent bud development and transcriptome in the grapevines *Vitis riparia* and *V.* spp. 'Seyval' (Seyve Villard 5276) was studied to separate bud maturation processes from dormancy induction responses. Photoperiod treatments were imposed 35 days after budbreak and transcriptomic analysis was conducted during the subsequent 42 days of bud development. Buds in both photoperiods were floral competent by 21 days of treatment (56 days after budbreak) but there was faster floral meristem development in LD than SD buds. Genes with a potential differential role in meristem development or dormancy transitioning were identified using differences in developmental stage between LD and SD buds and uncharacteristic gene expression trends in relation to bud meristem development.

P18

THE BEAUTY OF NAKED GRAPE BERRY CELLS

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Almost 50 years ago, E.C. Cocking demonstrated in Nature that naked cells called protoplasts might be obtained through enzymatic degradation of cell walls (Cocking 1960). Since then, as single cell systems, protoplasts have been used in physiological, biochemical and molecular studies aiming the investigation, improvement or modification of plants. In grapevine, protoplasts have been isolated from leaves, stems, roots, callus and embryogenic tissue, but their incapacity to express the totipotency has limited their utilization in breeding programs (Papadakis *et al.* 2009). However, grape berry protoplasts have not been achieved yet, a major challenge given the uniqueness of grape fruit for human diet and wine production. Also, as ripe grape berry has been considered a 'small bag of sugary water' without cell compartmentation and/or membrane integrity, the isolation of intact cells from the mesocarp is of special scientific significance. Protoplasting from grape berry mesocarp was achieved with cellulase and pectolyase followed by differential and gradient centrifugations (Fontes et al., 2009); however, given the special characteristics of berry tissue, cell wall digestion and protoplast purification were performed in a special environment to maintain their integrity and viability. Light, epifluorescence and confocal microscopy revealed the spatial organization of the cytoplasm where an intricate acidic vacuolar apparatus predominates. The fluorescent probes FM1-43 (labelling of the membrane apparatus), Fluo-4 AM (to show calcium accumulation in the vacuoles), DAPI (nucleus) and MitoTracker Red (mitochondria) where used. Purified vacuoles where stained with FM1-43, Fluo-4 AM and Neutral Red. Copper sulphate is widely used as an antifungal reagent for grapevine protection, but it may have deleterious effects for the vine and the wine. We have shown that copper sulphate negatively affects the viability of grape berry protoplasts in a dose-dependent manner and clues for the involvement of a Cu^{2+}/H^{+} antiport system on cation sequestration in the vacuole were obtained. Following the worldwide economical and social importance of wine in modern days, grape berry protoplasts are a major advance for both basic research of fruit ripening and biotechnological applications. The present work clearly supports the idea that berry softening during ripening is not strictly associated with loss in compartmentation and/or membrane integrity, confirming the recent work of Krasnow et al. (2008) that was based on the assessment of cell viability in fruit discs with fluorescein diacetate.

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P19
REGULATED-DEFICIT IRRIGATION RESULTS IN CARBOHYDRATE METABOLISM-ASSOCIATED ALTERATIONS IN GRAPE BERRY

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Irrigation is being used by modern viticulture as a way to maintain yield and improve wine quality when facing drought conditions. Nevertheless the controversy over this practice still exists. Excessive water can reduce berry quality traits such as colour, acidity or sugar content and promote an excessive vegetative growth. One way to reduce these detrimental effects is through water-management regimes such as regulated deficit irrigation (RDI). In RDI water is supplied at specific periods of the crop cycle which allows controlling vegetative vigour and also fruits size and quality. A key contribution to the improvement of grape wine quality under irrigation is to understand the underlying mechanisms that regulate grapevine fruit development and maturation as a gap of knowledge still exists in this field. Grape berry exocarp (cv Aragonez) transcriptome and proteome were studied. Three conditions (full irrigated, regulated deficit irrigated and rain fed non irrigated grapevines) were imposed from the green berry to the full maturation stage. A comprehensive microarray analysis of transcriptional changes was undertaken using a 23K custom Affymetrix GeneChip (developed by GRAPEGEN Project, Lijavetzky e*t al*. in preparation). Total protein skin extracts were analysed by two-dimensional gel electrophoresis (2-DE). Among the differentially expressed protein/genes water availability influenced the pattern of expression of several carbohydrate associated enzymes. Furthermore, stress and developmental responsive alterations were identified and the main results are discussed in relation to the physiological response of Aragonez to irrigation. Altogether, the analysis of transcriptomic and proteomic data provided a broad overview of the differentially expressed genes and proteins associated with grape berry fruit maturation under different water availability conditions.

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P20

TRANSCRIPTOMIC ANALYSIS OF TRANSGENIC GRAPE (*Vitis vinifera*) PLANTS EXPRESSING PGIP

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To prevent and control *Xylella fastidiosa*, the causative agent of Pierce's disease (PD), grapevines of *Vitis vinifera* cv 'Thompson Seedless' (TS) were transformed with the pear polygalacturonase inhibiting protein (pPGIP), a stimulator of plant innate immunity (Dandekar *et al.* 2008). Transgenic lines were obtained using *Agrobacterium*-mediated transformation (Agüero *et al.* 2006). Transgenic plants expressing the PGIP protein show decreased symptoms in leaves after infection with *X. fastidiosa* and with *Botrytis cinerea* (Agüero *et al.* 2005). Microarray (Affymetrix) analysis of leaves from a PGIP expressing line TS-50 and non-transgenic line TS showed that 3007 genes were significantly up- or down-regulated (FDR-adjusted p-value cut-off $p < 0.05$) in the transgenic line. These genes were further examined for function using the MapMan program that permits visualization of metabolic pathways based on over 900 functional classifications in *Arabidopsis* (TAIR 7). Many of the differentially regulated grapevine genes are homologous to *Arabidopsis* proteins from the cell wall or from secondary metabolic pathways related to plant defense. Additional gene annotation with the Gene Ontology vocabulary has been conducted using BLAST2GO. Transcription of pPGIP and other differentially expressed transcripts have been validated using RT-qPCR with TaqMan probes to identify the most important affected pathways. Leaf extracts from transformed and wild type TS plants were tested for polygalacturonase-inhibiting activity using polygalacturonase obtained from *B. cinerea* isolated from grape. Selected transformed lines have been bench grafted with wild type TS scions. Preliminary results showed that the PGIP protein moves from the rootstock up into the xylem sap of the wild type scion. Since pPGIP activity was found in the xylem sap of the untransformed scion when transgenic lines were used as rootstocks, we expect pPGIP will confer resistance to xylem-specific infections such as PD and assist in control of *X. fastidiosa* infections.

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P21

USEFULNESS OF BIOREGULATORS AND *CYANOBACTERIA* IN THE IMPROVEMENT OF THE VINEGRAPE PLANT DEVELOPMENT AND HEALTH STATUS UNDER STRESS CONDITIONS

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Bioregulators and *Cyanobacteria* positively affect the grapevine plant growth and development under drought and temperature stress conditions. Soaking of the hardwood cuttings in Asahi SL, Biochikol 020 PC, Tytanit, Citrosept, Biosept or watering of them with *Cyanobacteria* positively affect their rooting and increase the length and number of canes in plants, number of internodes, chlorophyll $_{(a+b)}$ content, activity of phosphatase, RN-ase and total DNA content. The positive effect of the applied biostimulators on plants is prominent under optimal growth conditions and in alleviation of the adverse effects of temperature and drought stress. Asahi SL (0,2%; sodium ortho- and para-nitro phenolate, sodium 5 nitro guaiacolate; Asahi Chemical Mfg. Co. Ltd., Japan) application to the cuttings before rooting is the most beneficial among all applied bioregulators. The positive reaction of its application is remarkably prominent when cuttings are exposed to the most severe drought stress at the beginning of rooting. Asahi SL increase also plant resistance to frost and positively affects their further growth in the next vegetation seasons. Biochikol 020 PC (1%; chitosan; GUMITEX, Sp z o.o., Poland) alleviates adverse effect of stress mainly when cuttings with more advanced rooting are exposed to the drought. Application of Biochikol 020 PC is also advantageous in alleviation of temperature stress (10°C). Tytanit (0,2%; titanium; INTERMAG, Poland), increases root system development, length of canes, number of canes or internodes and health status of plants subjected to drought stress. The positive effects remain also visible after one year of Tytanit application. Irrespective of the optimal or drought stress conditions Citrosept and Biosept (0,2%; Vitamin C, bioflavonoids; CINTAMANI, Poland) positively affects grapevines rooting and plant development in concentration of 0,2%. Citrosept is more effective in respect to the number of canes, while Biosept positively influences the length of canes. Cyanobacteria advantageously affects plant development and some metabolic processes, including chlorophyll $_{(a+b)}$ content, total DNA content and acid (pH 6,0) or alkaline (pH 7,5) phosphatase activity. The most beneficial effect is observed due to *Anabaena variabilis* and *Microcystis aeruginosa* (Trebon) watering of the rooted cuttings or plants.

Very effective in increasing plant development and in alleviating of the adverse effects of temperature, drought and pathogen stress are biostymulator Physe (containing oligosacharides), EM (mixture of antagonistic effective microorganisms) and AQ 10 (containing fungi *Amphelomyces quisqualis*). Watering, spraying or watering and spraying with these bioprotectors increase growth of plants and their health status. This treatment decrease infection of plants by *Uncinula necator Botrytis, Alternaria, Verticillium, Cladosporium, Fusarium, Aspergillus* and *Penicillium*. Treatment with effective microorganisms (EM) is most effective. The increased plant development is associated with the higher chlorophyll $_{a+b}$ content and increased photosynthesis activity in leafs.

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P22
ANALYSIS OF PINOT VARIETIES BY MICROSATELLITE MARKERS

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Molecular markers are widely used because of the wide range of applications. The identification of the varieties and clones of Pinots by microsatellite method according to the literature, present difficulties (Regner *et al*. 2006, Stenkamp *et al*. 2009). Hocquigny et al (2004) carried out SSR analysis between Pinots. Polymorphism analysis between 'Pinot' clones revealed that 65% shared the most frequent genotype. Moreover, the variant clones showed at least 96% similarity with this genotype.

Based on our former experiences 7 Pinot gris, 4 Pinot noir clones and Pinot blanc were analysed in 16 (VVS2, VMC5E9, VMC3D12, VVIM10, VMC5G8, VMCNG1E1, VMC1F10, VMC2H4, VMC8A7, VMC7G3, VVMD28, VrZag21, VrZag79, VMC1C10, VrZag25, Scu06vv) microsatellite loci.

A dendogramm (Figure 1) was constructed using Jaccard index for the estimates of genetic similarity between pairs, and average linkage for clustering.

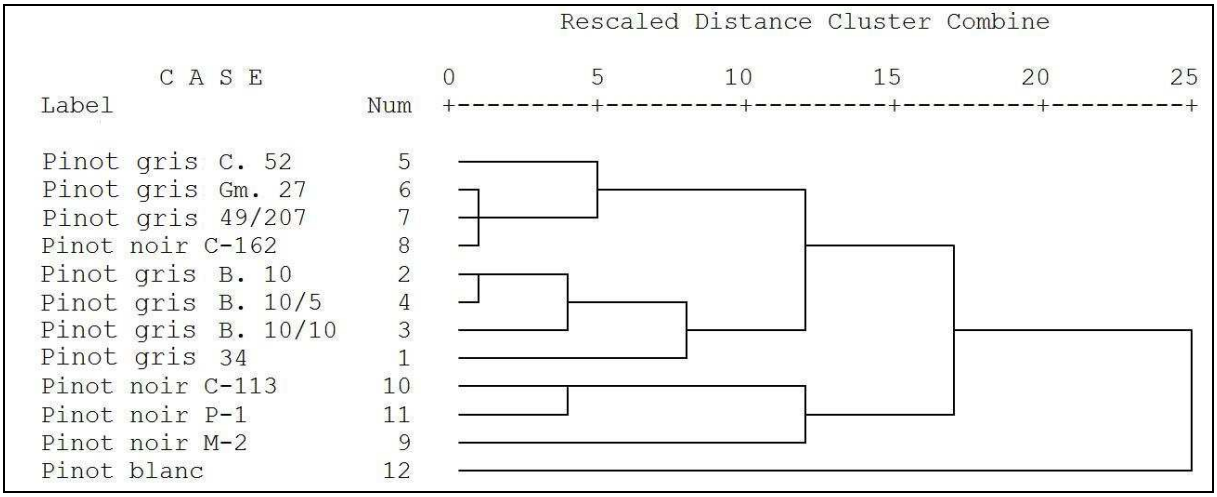

Figure 1. Dendogramm of Pinot clones based on SSR data.

Based on our results, it can be established, that the Pinot clones all showed high similarity. The Pinot gris clones bred in Badacsony, Hungary (B. 10, B. 10/5, B. 10/10) formed a group and showed the highest similarity with Pinor gris 34 from Romania. The other Pinot gris clones formed another group with Pinot noir C-162. These clones all originated from western Europe (Germany, France). These genetic differences could be traced back to the different geographical origin of the different clones.

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P23

GENETIC BACKGROUND OF THE ROOTSTOCK BREEDING AGAINST ABIOTIC STRESS FACTORS

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Grapevine rootstock breeding has started in the 1970-s at the Georgikon Faculty of Pannon University on the base of the rootstock collection consisted 118 genotypes. The main aim was to establish highly lime tolerant rootstock, because closely half of the Hungarian vine districts suffered from that problem even to use Teleki's originated rootstocks. First the Teleki's originated rootstocks have been classified not only morphological characteristics, but lately on the DNA base also. In a second step the crossing partners were chosen for the breeding aims. Meanwhile another breeding aim was added as drought tolerance increase. Screening of the rootstocks had been started for crossing partners in mid 1980-s. Seedling populations were made in mid 1990-s. The evaluation of individuals is in process. Seedlings were evaluated morphologically at first and later on the genetic characterization has started on 96 genotypes (93 rootstocks and 3 Vitis vinifera L. varieties) with 19 SSR primers. The primers had been chosen from each chromosome to give well defined heterozygosis and chance to established connection map with lime and drought tolerance characteristics. The preliminary results of the SSR analysis show high heterosigosity and polymorphism among the genotypes, the ranges of the SSR fragment length are more or less different in rootstocks and in Vitis vinifera L. varieties.

P24

HEAT AND LIGHT INFLUENCE ON GENE EXPRESSION AND METABOLITE ACCUMULATION IN GRAPE BERRIES UNDER DIFFERENT MICROCLIMATE CONDITIONS

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High temperatures due to global climate change may deeply modify the metabolic composition of grape berries. In addition to warmer mean temperatures along the year, climate change also shortens the phenological development of flowers and fruits, which induces a shift of the ripening phase under warmer summer temperatures. We studied the effect of defoliation, sun-exposition and east/west orientation on grape berries after veraison. Two weeks after defoliaton, there is no difference in berry sugar content, but flavonol and anthocyanin contents are significantly altered. Gene expressions were assessed for sun-exposed berries on the west side showing significant differences of flavonol and anthocyanin accumulation with non-exposed berries on the east side. Sampling was made 0,1,4,7 and 14 days after defoliation. We used full genome microarrays produced with a Combimatrix synthesizer and based on the grapevine genome (Jaillon et al. 2007). Using stringent statistical methods, about 50 differentially expressed genes were identified. Some of them closely correlate with cumulated berry temperature difference after defoliation or with daily berry temperature difference between the two conditions.

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P25

VITIS VINIFERA CV. TOURIGA NACIONAL AQUAPORIN GATING STUDIES USING SACCHAROMYCES CEREVISAE IN COMBINATION WITH A STOPPED-FLOW TECHNIQUE

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Water availability is of fundamental importance for all living organisms. To cope with environmental and physiological stresses, plant, as all other organisms, must be able of a rapid cellular adaptation for survival and growth. Depending on the environmental conditions and the water balance, plants can modify the relative contribution of apoplastic and cell-to-cell water-flow pathways across tissues to adjust the overall hydraulic conductivity. Aquaporins (AQP) are ubiquitary membrane channels with critical roles in controlling cell and tissue water fluxes. Due to their high isoform multiplicity in plants, elucidation of the physiological function of each isoform has been a difficult task. This can be overcome using yeast, which proved to be a suitable system for plant protein expression.

For a better understanding of *Vitis vinifera* cv. Touriga Nacional aquaporin function and stress defense responses, an important step is the characterization of these water transporters. We identified five putative aquaporin genes homologous to plasma intrinsic proteins (PIP2;2 and PIP1;1) and tonoplastic intrinsic protein (TIP2;1). Heterologous expression in a *aqy1aqy2* double deletion *S. cerevisiae* strains lacking aquaporin activity has been performed and chimeric GFP-aquaporin fusion proteins showed their localization in the plasma membrane. Aquaporin function was assessed in intact transformant yeast cells by imposing osmotic gradients of an impermeant solute and following the time course of water fluxes in a stopped-flow fluorescence device. Moreover, regulation of grapevine aquaporin by a gating mechanism involving its protonation under cytosolic acidification was also investigated.

The osmotic permeability coefficients (Pf) obtained for yeasts expressing individually PIP2;2 and PIP1;1 were similar to the double deleted aquaporin strain (with high activation energy (Ea) for water transport), suggesting a non-significant water transport route through these channels under the tested conditions. However, for TIP2;1, Pf increased up to 4.5 fold (lower Ea) compared to the values obtained when water transport occured mainly through the lipid bilayer. Our results also evidenced a possible intracellular pH dependent aquaporin regulation.

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P26

CHARACTERIZATION OF THE ROOTSTOCK EFFECT ON THE RESPONSE OF VINE TRANSPIRATION DURING A DROUGHT CYCLE

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Background and aims: Water is the main limiting factor for yield in viticulture. Vine water status also strongly impacts grape quality. Studies concerning vine water deficit stress are numerous but the level of water stress is seldom rigorously controlled. Drying cycles used in other woody species allow neither to know exactly the intensity of water stress nor vigour differences. The aim of this work was first to define a method for applying the same gradual water stress to all the individuals of a pot experiment, whatever their leaf area. Second, the response of transpiration induced by different rootstocks was characterized.

Methods and results: Young grafted vines of Cabernet Sauvignon were grown in 7L pots filled with a known amount of sandy-loamy soil. The rootstocks studied were *V. riparia* "Gloire de Montpellier", *Vitis* hybrid 110 Richter and several hybrids *V. vinifera* Cabernet Sauvignon * *V. riparia* Gloire de Montpellier. Water retention properties of the substrate were primarily determined. The amount of water in the substrate was used to monitor soil water status. Transpiration was evaluated daily by weighing each pot individually. Irrigation was applied in the mid morning in order to compensate exactly the difference between the daily water loss due to transpiration in a particular pot and the loss of water of the least transpiring plant. Leaf area measurements were performed regularly in order to calculate the daily transpiration per units of leaf area.
A progressive water limitation occurred. Daily water status of the substrate was expressed as the amount of water still present in the pot. Transpiration was stopped within 40 days. Normalized daily transpiration data per unit of leaf area were plotted with percentage of water retention capacity. Some specific parameters of these relationships were used to compare various rootstock genotypes. The threshold of water content corresponding to the onset of regulation for vines was calculated to characterize the various rootstocks. This threshold presented significant differences between the studied rootstocks. 110R presented a significantly lower threshold than the others in two different experiments run in 2006 and 2007.

Conclusions: Thanks to this method, every plant faced the same water stress at a daily scale. This control allows more accurate comparisons of the water extraction capacities for different rootstocks.

Significance of the study: This method may be useful to compare vine rootstock performances related to water availability.

P27

FOUNDING A SWEDISH VINEYARD AND WINERY

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The aim has been to assess the potential for an economically successful wine production in Sweden. Production costs for cultivating and producing wine on a farm in Sweden were calculated. Depending on yields, the estimated costs varied from Euro 15.1 for production of 1 800 litres wine per ha and year to Euro 41.9 for 525 litres per ha and year. For annual production of 1 800 litres per ha, which could be achieved when the vineyard is established, the capital requirement was Euro 730 000. It would take six years for the investment to be paid off if the wine is sold for Euro 37.5 per litre to the distributor/wine merachant. If the vineyard forms part of general farm operations, the work periods can be optimised. Wine produced in northern Europe has the advantage that the cold nights and long hours of daylight give more aromatic grapes of a somewhat smaller size, leading to a more concentrated grape must. Since yield per unit area is low, the price of the Swedish-produced wines cannot compare in the standard market segment. A high price can be justified if an image has been built up around the wine and if the product is of high quality. Selling a wine that costs over Euro 10 to a customer in Sweden is considered to be a question of selling an experience, not a beverage. The strategy of striving for a high quality product must be defined before starting the vineyard and winery.

P28

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF A bHLH TRANSCRIPTION FACTOR INVOLVED IN FLESHY FRUIT RIPENING

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Grape berry development can be divided in two phases: a phase of "herbaceous" growth mediated by cell division and elongation. At this stage, fruits are green, acid and hard. The second phase corresponds to cell elongation and is characterized by the maturation of berries which is typified by changes in texture and colour. This transition from green stage to ripening is called véraison. From véraison on and during ripening, high amounts of sugars and phenolic compounds accumulate in berries. In addition to their structural role, sugars can act in various signaling processes, thus being able to affect fleshy fruit development. In this context, we identified a new transcription factor, VvbHLH1, which is regulated by sucrose and exclusively expressed in the berry. Its functional characterisation suggests that VvbHLH1 may play a key role during berry development and ripening.

P29

DIFFERENCES IN WATER USE EFFICIENCY OF TWO *VITIS VINIFERA* L. CULTIVARS DURING DROUGHT AND RECOVERY

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A comparative study on water-use-efficiency (WUE) under water deficit and recovery was conducted on grapevines of the cultivars Grenache, of Mediterranean origin, and Syrah of mesic origin, grown in Mallorca (Spain). The experiment was performed from June to August 2008 at the "Universitat de les Illes Balears" (Mallorca, Spain). Deficit irrigation was established according to the leaf maximum daily stomatal conductance (g_s), to achieve a severe water stress in one week treatment. After one week under those conditions, all plants were irrigated to field capacity.

Leaf photosynthesis and transpiration measurements were taken daily, while another physiological measurements as midday leaf water potential and hydraulic conductivity were performed only on five specific sampling days per each treatment: the day the desired stomatal conductance (50 $mmolm^{-2}s^{-1}$) was first achieved (day 0), seven days after sustaining the plants at constant soil moisture, just before re-watering (day 7), and then 1, 3 and 7 days after re-watering,. The carbon isotope ratio in leaf dry matter ($\delta^{13}C$) was measured during water stress, 7 days after acclimation and after re-watering.

The goal was to analyze how stomatal and mesophyll conductance are regulated under water stress and recovery, as well as how water stress adjustments affects the leaf water-use-efficiency (WUE). Water stress induced a substantial stomatal closure and important reductions of gm at time with an increase in the WUE, which interestingly persisted many days after re-watering. Syrah maintained lower maximum stomatal conductance (gmax) and maximum leaf photosynthesis (Amax) values than Grenache at lower leaf water potentials throughout the season. The gm dynamics showed interesting differences in respect to g_s both under water stress and mainly for the recovery. Kh showed important reductions under water stress, and a slow recovery after irrigation.

Leaf WUE increased in response to water stress but was severely reduced during the recovery. Differences in plant WUE were also reflected in leaf $\delta^{13}C$ values.

P30

BOTRYTIS IN ORGANIC VITICULTURE

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Organic growing of grapes has increased in Austria in the last years to 6% of the viticulture area. The change to this production method is political encouraged and financially supported by money from the ÖPUL program.
In organic viticulture Botrytis is the most serious threat for the production due to a lack of appropriate protection treatments. Therefore numerous activities have been started to develop methods to inhibit grey mould of the grapevine. We screened various substances on Petri dishes to verify the effect of suppressing growth. Finally in field trials the following substances were tested: Extract of fennel, extract of Fallopia japonica (Milsana) extract of sage, potassium water glass, Bacillus subtilis, Aureobasidium pullulans, Trichoderma, Armour Zen, Botry Zen, Cocana, and others.
Some of these products are commercially available and others are under development. They were evaluated for their efficiency in protecting grapes. The extent of protection was in some cases to low to become a tool for the organic production. Nevertheless methods with physical effects on the bunch as partitioning of bunches or wiping off the flower show convincing effects. This methods directed under cooler flowering weather conditions, as we had it this June, to very loose clusters with small crop quantities. Other possibilities for the organic production could be the use of clones with looser cluster formation or new crossed varieties with higher resistance against Botrytis.

P31

CLIMATE CHANGE-MEDIATED CHANGES IN PHOTOSYNTHESIS, WATER USE EFFICIENCY, AND BERRY MATURITY OF GRAPEVINE (*Vitis vinifera* L.) CV. TEMPRANILLO.

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Greenhouse experiments were conducted to investigate the impact of predicted climate change (elevated CO_2, 700 µmol CO_2 mol^{-1} air vs. ambient; elevated temperature, 28/18°C vs. 24/14°C, day/night; and moderate drought, 40% of field capacity vs. well-irrigated) on photosynthesis, water use efficiency and berry ripening from veraison to full maturity. Grapevine (*Vitis vinifera* L. cv. Tempranillo) fruiting cuttings were used as experimental plant material. At harvest time, grapes from climatic change-related treatments had higher ºBrix, indicating a shortened ripening time. Grapevine fruiting cuttings responded to elevated CO_2 and temperature increasing photosynthetic rates. These higher photosynthetic rates were related to increases in leaf CO_2 availability, which was reflected in markedly higher sub-stomatal CO_2 concentrations. Also, total chlorophyll concentrations, mostly due to chlorophyll *a*, and relative water content increased at harvest time in high CO_2 and elevated temperature treatment. Stomatal conductance and transpiration were markedly reduced in the climatic change simulation treatment when compared to the control one, due to stomatal closure. Plants under high CO_2 and temperature during the ripening process fixed more CO_2 per transpired water unit than the treatment with ambient conditions, improving plant photosynthetic water use efficiency. Changes in photosynthesis possibly explain the higher ºBrix values of climatic change-exposed plants, increasing grape sugar levels at harvest time.

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P32

EFFECTS OF FROZEN STORAGE ON RED WINEGRAPE QUALITY PARAMETERS

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Frozen-storage of samples is often required at laboratories assessing grape quality as, at certain moments, the quantity of samples to analyse can go beyond the possibilities of either the facilities or the staff. However, there is little information on how freezing and thawing may affect grape composition due to water loss, contamination, physical and chemical reactions or component degradation (Cynkar *et al*. 2004, 2009).

The aim of this work is to evaluate the effect of short-term frozen storage (1 month) on the main quality parameters of 'Cabernet Sauvignon', 'Tempranillo' and 'Garnacha'. 50 samples were collected during the 2009 vintage in Navarra (Spain), at different degrees of ripeness in order to cover a broad range of situations. 20 entire bunches per sample were carried to the laboratory at low temperature, all their berries hand plucked, and two 200 randomly taken berry subsamples kept. Half the subsamples were immediately homogenised, and total soluble solids (ºBrix), pH, titratable acidity, malic and tartaric acid, yeast available nitrogen and total and extractable antochyanins determined. The remaining subsamples were frozen at -18ºC for one month and, after overnight thawing at 4 ºC, analysed following the same procedures. The results obtained in fresh analyses and after frozen storage were compared through linear regression. In this contribution, the effect of short term frozen storage will be discussed.

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P33

SUITABILITY OF PRE-DAWN AND STEM WATER POTENTIAL AS INDICATORS OF VINEYARD WATER STATUS. A STUDY-CASE FOR CV. 'TEMPRANILLO'

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Under arid and semi-arid climates water availability is frequently the most limiting factor for vineyard productivity, since water deficit reduces yield and sugar accumulation, and affects grape quality. Proper irrigation management requires reliable tools that allow the growers to make decisions rapidly and in a simple way. The most frequently used methods are (i) estimation of water consumption from climatic data, (ii) measurement of soil water content or availability and (iii) measurement of plant water content or of (iv) plant activity. One of the tools preferred by scientists and, increasingly, by growers is Scholander pressure bomb, that allows a relatively quick, flexible, and accurate estimation of plant water status through the measurement of leaf water potential. Pre-dawn (Ψpd) and stem (Ψs) water potential have quite a clear physiological significance. Although under some circumstances Ψpd, Ψs, and even Ψl have been reported to be almost equivalent, that is not the general trend, and there is no agreement among viticulture researchers on which of those measurements allows estimating vineyard water status best. The aim of this work is to shed some more light on this subject, comparing the performance of measurements of Ψpd and Ψs (at mid-morning and midday) under a wide range of water statuses and vineyard conditions for 'Tempranillo' in a semi-arid climate.

Seven different sites in Southern Navarre, Spain were used in this experiment, in an area characterized by a semiarid climate. Ψpd and Ψs (mid-morning and noon) values obtained at each vineyards were compared through regression analysis considering climatic conditions. Data were taken in 2007, 2008 and 2009. The obtained results show that, although Ψpd and Ψs at mid-morning and at noon are related, fruit load, soil depth and slope orientation may affect the relationships, i.e.: stem water potential dynamics along the day. The implications of these results for research and irrigation management decision-making will be discussed.

P34

ELICITATION OF GRAPEVINE DEFENCE RESPONSES AGAINST DOWNY MILDEW CAUSED BY *PLASMOPARA VITICOLA*

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Abstract:
Plasmopara viticola the casual agent of downy mildew is one of the most destructive grapevine diseases in Europe and in the eastern half of the United States. In the last decades, induced resistance to *P. viticola* has gained increasing attention as a strategy to control the disease in a more sustainable way.
This work aims at triggering the plant's own defence mechanism/s against *P. viticola* by some selected elicitors, through prophylactic as well as curative strategies. For evaluating the level of induced resistance, an integrated approach will be followed to assess the efficacy of the used elicitors as well as to understand their mode of action. This includes synthesis of phytoalexins as well as PR-proteins, cell death (hypersensitive response) and defense gene and protein expression, to name a few.
So far disease severity as well as disease development has been assessed. Callose formation was measured as an indicator for the activation of defence reaction. First experiments indicated different infection intensities as a result of different elicitor applications indicating that some elicitors had an effect on reducing the spread of the disease. While for disease development, haustoria formation, oospore germination and hypersensitive response was measured using epifluorescence microscope.

P35

GRAPE CLONAL CHARACTERIZATION TACKLED BY GENOME-WIDE ANALYSES

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Grapevine (*Vitis vinifera* L.) is a long-living and woody perennial plant grown worldwide. Vegetative propagation over long periods favours the accumulation of mutations within individual genotypes, which exhibit altered phenotypes. Clonal selection as a procedure of crop improvement takes advantage of the identification of sports with agronomically and enologically important traits, which are vegetatively propagated resulting in new grape clones. Given the high economical value of clones, their identification is of great relevance and in addition it pertains issues of patenting and legal rights. While cultivar identification in grapevines is traditionally based on ampelographic descriptors and on microsatellite (SSR) profiles, clone discrimination is not possible with such tools. To allow true genetic identification of clones, molecular marker systems need to be developed ad hoc. Given the availability of the Pinot Noir (clone ENTAV 115) genome sequence, it is timely to use techniques exploiting the polymorphism information of unique coding and non-coding regions along with approaches based on specific genomic sequences of interest, such as DNA transposons and retrotransposons. Transposable elements (TEs), which possess the capability to change their genomic location, are potential source of mutations leading to clonal variation. In this study we focus on the application of two genome sequence based approaches, SNPlex™ Genotyping System and Transposon Display, to tackle clonal characterization within six wine grape cultivars. We have analysed the state of 573 putative (electronic) SNPs, identified in coding and non-coding regions of the mentioned grape genome, in 141 genotypes. This sample set refers to 3 biological replicates (plants) of 47 clones (both registered and biotypes) belonging to the Pinot Noir, Pinot Gris, Pinot Blanc, Meunier, Teroldego and Gewürztraminer cultivars. The same set of clones was tested with 17 primers targeting specific regions (LTRs, LTR downstream or upstream, ORF) of TEs belonging to 6 families. Here we report preliminary results about the polymorphisms identified by different approaches enabling molecular characterization of clones within international and local grape varieties.

P36

EFFECT OF OSMOTIC STRESS AND POST-STRESS RECOVERY ON THE CONTENT OF PHENOLICS AND PROPERTIES OF ANTIOXIDANTS IN GERMINATING SEEDS OF GRAPEVINE *VITIS CALIFORNICA*

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The tested material consisted of grapevine *Vitis californica* stratified seeds germinated under optimum conditions (+25^{o}C in water), under osmotic stress (-0.2MPa w PEG solution) and submitted to recovery after stress (+25^{o}C in water). The germinating seeds were determined to contain tannins, catechins and the following phenolic acids: gallic, caffeic, *p*-coumaric and ferulic. The acids occurred in free, ester- and glycoside-bounded forms. The dominant form of phenolic acids was the ester-bounded fraction. Gallic acid was the most abundant phenolic acid in germinating seeds, while ferulic acid appeared in the smallest amounts. Our analysis of tannins demonstrated that osmotic stress depressed their concentration. Presence of catechin group compounds such as catechin and epicatechin was also determined. In each sample epicatechin was dominant. The total concentration of catechin increased under stress conditions and declined during post-stress recovery. Catechins are a constituent of tannins and their increase under osmotic stress is most probably caused by the breakdown of some tannins in seeds germinating under stress conditions. Samples submitted to osmotic stress were also found to contain less of total phenolic compounds, whereas in samples which underwent post-stress recovery the total level of phenolic compounds increased. Compared to extracts from seeds germinating under optimum conditions, osmotic stress depressed the capacity of extract to scavenge $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radicals, but the antioxidant activity rose in seeds submitted to recovery after stress. Positive correlation was therefore demonstrated between the total content of phenolic acids in germinating grapevine seeds and the reducing power of extracts obtained from these seeds and their free radical scavenging activity. The results suggest that osmotic stress inhibits the activity of secondary metabolism enzymes in germinating grapevine seeds. Thus, the antioxidative defence system is largely blocked under osmotic stress. It seems that a very high oxidoreductive potential in grapevine tissues prior to occurrence of osmotic stress is essential for maintaining proper homeostasis of oxidation and reduction reactions.

P37
THE INFLUENCE OF ETHYLENE POSTHARVEST TREATMENTS ON QUALITY-RELATED METABOLIC PROCESSES IN WINE GRAPE BERRIES

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The goal in postharvest treatments of some fruit is often to delay the ripening process. However, in wine grapes, the target is to concentrate metabolites and get a more advanced/balanced maturity, especially in terms of phenolic compounds. This has been observed during berry dehydration (for the production of special wines such as passiti) and can be performed in tunnels (Rizzini, et al., 2009). These tunnels permit gas treatments that could be effective in modulating metabolic processes and, as a consequence, the composition of the berries and the quality of the wines. The effects of ethylene treatment at veraison have been studied by a large-scale transcriptomic analysis via microarrays (Chevrin, et al., 2008), but little is known about postharvest physiology of wine grape berries especially in the presence of the gaseous hormone. A large-scale transcriptome analysis, using an Comibmatrix microarrays 30 K (29 290 probes in triplicates) on skins of Sangiovese grape berries subjected to 1000 ppm ethylene for one day after harvest has been performed. After statistical analysis (SAM with the MeV program), we found 3159 down regulated and 2595 up regulated targets at a cut off of 20% following both treatments, indicating that ethylene induces marked changes in transcriptome after harvest in a non-climacteric fruit such as grape berries. In fact, several ethylene responsive genes were modulated indicating that a detached grape berry is indeed sensitive to ethylene. Gene Ontology (GO) analysis revealed that the largest group of genes falls in the oxidation reduction class (151 genes). Another effect of the presence of ethylene is the activation of a significant number of genes that are involved in the cell wall degradation. The degradation of the cell wall was measured in the following harvest on different samples of Sangiovese skins by the extractability index. The extractability index being lower for the treated samples indicates indeed a higher degradation due to the ethylene treatment. With respect to the phenolic profile, we performed the spectro-photometric and colorimetric essays for the major classes of phenols and HPLC screening for the major phenols found in grapes. Both assays gave similar trends and results when berries treated for 1 and 2 days with ethylene followed with 2 days of dehydration were analyzed. Unlike the control for which the phenols decreased after detachment, the ethylene allowed the phenolic content to remain constant. Anthocyanins in both treated and control samples decreased but ethylene lessened the decrease. Ethylene was more efficient after one day of treatment on simple anthocyanins, but 2 days of treatment gave better results for acylated anthocyanins (acetyl and p-coumaryl compounds) as they returned to the level detected at harvest. Acetylation is known to promote condensation and thus improve wine stability, although this process also occurs during wine aging: the earlier it occurs, the more anthocyanins are preserved. In conclusion, ethylene treatment seems advantageous since it compensates for the oxidation of phenols that is initiated after fruit detachment and induces structural changes of cell wall resulting in improved extractability during maceration

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